Biomarkers of Alpha Particle Radiation Exposure

Prepared by:
Dr Vinita Chauhan
Health Canada

Scientific Authority:
Norman Yanofsky, 616-944-8161
CBRN Portfolio Manager, DRDC Centre for Security Science

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IMPORTANT INFORMATIVE STATEMENTS

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Abstract

The threat of terrorist-precipitated nuclear event places humans at risk for radiological exposures. Increased forensic capability through the development of biological tools to help identify those involved should be an integral to a national strategy against terrorism. Such capacities may potentially deter a mass casualty event and would be of benefit for rapidly identifying those who are exposed, if such a terrorist event were to occur. Among the isotopes to be used, those emitting alpha-particles pose the highest risk. Here we describe work towards the identification of gene-based biomarkers of alpha-particle radiation exposure. Peripheral blood mononuclear cells (PBMN) isolated from healthy individuals were irradiated with alpha-particle radiation at doses of 0, 0.5, 1.0 and 1.5 Gy. Genomic strategies were then employed to identify transcripts that were differentially expressed relative to un-irradiated cells, 24 hours post-exposure. Stringent statistical analysis identified strong responding genes at each of the individual doses tested. Among these genes, 31 were common to all doses with high expression levels ranging from 2-10 fold. This subset of genes were further validated in complete white blood cell (WBC) populations and compared to X-ray exposure using quantitative real-time PCR. The gene panel was responsive in the alpha–particle exposed WBC's and was shown to exhibit a unique expression profile from X-irradiated cells. This initial data is promising and has provided a sub-set of dose-responsive genes to radiation exposure which may provide a means for developing detection strategies for special nuclear material handling and exposure in humans.

Résumé

La menace d'un événement terroriste nucléaire précipité, place la population dans une situation à risque d`une exposition au rayonnement ionisant. Augmenter la capacité des laboratoires judiciaires, par le développement d`outils biologiques afin d`aider à l`identification de ceux qui sont exposés, devrait être une partie intégrante d`une stratégie nationale contre le terrorisme. Une telle capacité pourrait potentiellement dissuader de créer un très grand nombre de victimes et permettrait l`identification rapide des gens exposés advenant un tel événement terroriste. Parmi les isotopes utilisés, ceux émettant des particules alpha, posent le risque le plus élevé. Ici nous décrirons les travaux qui utilisent des bio marqueurs, basés sur l`identification de gènes associés à l`exposition au rayonnement ionisant causée par des particules alpha. À partir de sang périphérique de donneurs en santé, des leucocytes mononucléaires ont été isolés puis exposés à par des particules alpha à des doses de 0, 0.5, 1.0, 1.5 Gy. Des stratégies génomiques (24 heures post-exposition) ont été employées afin d`identifier des transcriptions exprimées de manière différentielle versus des leucocytes mononucléaires non exposés. Des analyses statistiques strictes ont permis d`identifier avec une réponse significative, des gènes à chacune des doses testées. Parmi ces gènes, 31 étaient communs à toutes les doses avec une réponse élevée entre 2 à 10 fois les valeurs des échantillons non-exposés. Ce sous-ensemble de gènes a été évalué de façon plus approfondie dans les leucocytes totaux. Pour ce faire, des analyses comparatives et

quantitatives de polymérisation en chaîne (PCR) en temps réel avec des échantillons exposés aux rayons-x, ont été effectuées. L'ensemble des gènes qui ont démontré une réponse positive aux particules alphas dans les leucocytes totaux ont aussi démontré un profil d'expression positif lorsqu' exposés aux rayons-X mais à un degré différent. Ces données préliminaires sont prometteuses et nous ont fourni un sous-ensemble de gènes exprimés lors de l'exposition au rayonnement ionisant. Ces résultats pourraient nous permettre de développer des stratégies de détection de matériel nucléaire spécial lors de la manipulation et l'exposition au rayonnement ionisant chez les humains.

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1 Introduction

Nuclear terrorism is an escalating global concern; our current capabilities to manage and prevent such a tragic event are limited. Since 1993-2011, the International Atomic Energy Association (IAEA) has documented 2164 nuclear material incidents or malicious acts and 588 involved the theft or loss of these materials. A further 18 involved plutonium or highly enriched uranium. Such illicit trafficking events highlight the potential for radioactive material falling into wrong hands and being used for the fabrication of a radiological dispersal device (RDD). Las Alamos National Laboratory has conducted a thorough review of RDD source material and has postulated that four of the nine isotopes most likely to be employed, are alpha-particle emitters. This is primarily due to their minimal shielding requirements and ease of concealment (Van Tuyle et al. 2003). In addition, there is also the advantage of their long half-lives and the resultant severe biological damage that can occur from a minimal dose of exposure.

Alpha-particle emitters are known to create very dense ionizing tracks as they traverse a medium. They can typically cause an energy deposition of 160 keV•μm-1 for 2.5 MeV alpha-particles in comparison to 2.0 keV•μm-1 for low linear energy transfer (LET) X-rays (Andreo, Evans et al. 2005). Therefore, alpha-particles produce more significant biological effects when compared to equal absorbed doses from low LET radiation, which are more sparsely ionizing (Nikjoo, O'Neill et al. 1999, Nikjoo, O'Neill et al. 2001). As alpha-particles have limited penetrating power, they are primarily of concern when inhaled, ingested or absorbed through an open wound. If ingested or inhaled, radionuclide's which emit alpha-particles may damage sensitive cells and internal human tissue (NRC, 1999). A number of *in vitro* and *in vivo* studies have shown that alpha-particle exposure can lead to mutagenic changes including large deletions, frameshifts and base-change mutations (reviewed in Jostes et al., 1996, Vral et al., 2011, Anderson et al., 2007).

There is currently limited available biological detection technology for determining exposure to alpha-particles and specifically in the detection of handlers of special nuclear material (SNM). SNM handling can elicit both internal and external biological responses. In the example of plutonium, direct exposure would cause a significant radiation dose to its handlers at the area of contact (i.e the palmar side of the hands). This would result in a unique spectrum of biomarkers that may serve as biological indicators of exposure. These biomarkers may then be procured from biological samples (i.e fingermarks, blood and saliva). Alternatively, there is also potential to inhale the emitted alpha-particles, resulting in internal contamination to organs, sensitive cells and blood. These biomarkers may be rapidly detected from a small sample of blood/saliva using quantitative polymerase chain reaction. This would also support high-throughput triage capacity in a mass casualty event.

To date, the majority of the bio-dosimetric methods are founded on cytogenetic changes resulting from DNA damage (Ainsbury et al., 2011). Such methods are time-consuming and require significant technical expertise. An alternative to these classic cytogenetic-based assays is the development of biomarker based bio-dosimetry, which has recently been put forth as a key priority area in the field of nuclear threat countermeasures. Microarray data / gene based profiling can serve as timely and minimally invasive procedure to support these needs. These technological advances have the capacity for monitoring changes in the expression of a number of genes following radiation exposure. The advantages are that there is potential to identify genes that may act as biomarkers of acute and chronic damage (Chauhan et al., 2011, 2012a,b,c,d,e). In addition profiles in gene expression may vary with respect to the radiation quality thereby providing a means for determining more accurate dose estimates for mixed radiation exposures.

In this R/N community development project, genomic strategies were employed to mine for robust transcripts that were modulated by alpha-particle radiation exposure. The most sensitive biological system to radiation exposure is the hematopoietic system, which is responsible for maintenance of peripheral blood and immune cell homeostasis (Stewart, Akleyev et al. 2012). Circulating hematopoetic cells were isolated from normal, healthy volunteers and subjected to *ex-vivo* exposure to alpha-particle radiation. Twenty-four hours post-exposure, the expression of 41,000 different transcripts were assessed using Illumina bead array technology. The gene expression profiles were compared to non-irradiated controls. Strong dose-responsive genes were then further validated in complete white blood cell population and assessed for specificity by comparison with X-irradiated cells. The results produced from this research have filled considerable knowledge gaps in the area of alpha-particle bio-dosimetry.

2 Purpose

The overall scope of this project is to develop and operationalize effective multi-purpose biological assessment tools for identifying special nuclear material (SNM) exposure. These tools would help in providing evidence for the prosecution of criminal acts or unauthorized uses of SNM and deliver advanced investigative technology necessary for the mitigation of a mass casualty event. The deliverables produced from this research will also support current triage capabilities following a radiological/nuclear (R/N) event or nuclear accidents such as Fukishima. It will help to identify individuals that have been exposed and will provide medical personnel biological dose estimates needed for proper medical intervention. Ultimately, it will enhance our existing bio-nuclear forensics capabilities and emergency preparedness response plans through the detection of those exposed to alpha-particle emitting radioactive material.

The specific goals of this community development study are:

1) The identification of gene-based blood biomarkers of alpha-particle radiation exposure and their associated pathways.

2) Preliminary assessment of the specificity of the responding genes by comparison with X-irradiated cells.

3) Preliminary assessment the ability of the gene panel to respond in a broader group of individuals.

3 Methodology

3.1.1 Blood Draws

All procedures were approved by Health Canada's Research Ethics Committee. Peripheral blood from healthy volunteers was drawn via periphery venipuncture with informed consent from all subjects and was drawn into either 5 x 10 ml EDTA (for gene analysis) or 2 x 4ml lithium heparin (for plasma analysis) vacutainer tubes (Becton Dickinson and Company, Franklin Lakes, NJ). A total of 6 male and 6 female donors participated. Before any further processing, a 100ul whole blood sample was drawn to perform a complete blood count (CBC) via automatic haemocytometer (Life Technologies, Burlington, On).

3.1.2 White Blood Cell and Peripheral Blood Mononuclear cell isolation

Peripheral blood mononuclear cells (PBMN) were used for the initial microarray screening. A similar isolation procedure was employed as described by Boyum in 1968. Briefly 15 ml of Histopaque-1077 (Sigma-Aldrich, MO, USA) was pipeted into upper chamber of Accuspin Tube (Sigma-Adrich). The tube was centrifuged (800 x g) for 30 sec. Freshly defibrinated whole blood was pipeted into the upper chamber of tube. The tube was centrifuged 800 x g for 15 mins. The band of mononuclear cells were transferred to an alternate centrifuge tube and washed with 10 ml of isotonic PBS three times and during the last wash, pelleted cells were resuspended in RPMI-1640 media supplemented with 10% FBS, L-glutamine and penicillin/streptomycin. For customized qRT-PCR experiments complete white blood cell (WBC) population was employed. White blood cells were isolated from whole blood using Histopaque-1119 (Sigma-Aldrich). Twenty-five milliliters of whole blood was gently poured over 15 ml of Histopaque-1119 (Sigma-Aldrich) and spun at 1000 x g for 10 minutes. This resulted in erythrocyte sedimentation below the Histopaque gradient and total white blood cell population above the gradient. This volume (~15 ml) was then transferred to a new 50 ml falcon tube and diluted 1:2 with phosphate buffered saline (PBS). The resulting white blood cell pellet was then washed twice more with 10 ml PBS and then resuspended in RPMI-1640 media supplemented with 10% FBS, L-glutamine and penicillin/streptomycin.

3.1.3 Plasma

Blood drawn into lithium heparin containers was spun down at 1000 x g for 5 minutes and then the top layer consisting of plasma was removed for experimentation. Aliquots of 1 ml were placed on Mylar dishes for exposure and were irradiated at 0.0, 0.5, 1.0, 1.5 Gy of alpha-particle radiation. Samples were frozen after exposure and stored at -40°C before being processed next day. Plasma was analysed using the Piccolo Express Chemistry Analyser (Fisher Scientific, Ottawa, ON) for *in vitro* diagnostics according to manufactures instructions. Table 1 provides a representation of typical values that were obtained from the ex-vivo irradiation of plasma with alpha particles from each of the individuals. Overall no significant effects were observed in each of the tested clinical biomarkers post-irradiation.

Table 1: A typical representation of clinical biomarkers from plasma obtained from healthy individuals. Plasma was ex-vivo irradiated with alpha particles (0-1.5 Gy) and assessed for expression changes of the various metabolites following irradiation.

Analyte	0 Gy	0.5 Gy	1.0 Gy	1.5 Gy
Alanine Aminotransferase (ALT) U/L	30	27	30	27
Albumin (ALB) g/dL	4	4	3.9	4
Alkaline Phosphatase (ALP) U/L	39	32	33	37
Amylase (AMY) U/L	46	43	43	45
Aspartate Aminotransferase (AST) U/L	44	41	39	42
Blood Urea Nitrogen (BUN) mg/dL	18	19	18	18
Calcium (CA) mg/dL	9.6	9.8	9.6	9.6
Chloride (CL-) mmol/L	101	103	100	100
C-Reactive Protein (CRP) mg/L	< 5.0	< 5.0	< 5.0	< 5.0
Creatinine (CRE) mg/dL	1.1	1.1	1.1	1.2
Direct Bilirubin (DBIL) mg/dL	0.3	0.2	0.2	0.2
Gamma Glutamyltransferase (GGT) U/L	28	26	26	28
Glucose (GLU) mg/dL	108	110	106	106
High Density Lipoprotein mg/dL	68	68	66	64
Low Density Lipoprotein mg/dL	172	164	170	167
Potassium (K+) mmol/L	4.5	4.6	4.5	4.4
Sodium (NA+) mmol/L	134	138	133	134
Total Bilirubin (TBIL) mg/dL	0.8	0.8	0.7	0.7
Total Carbon Dioxide (tCO2) mmol/L	29	29	31	30
Total Cholesterol (CHOL) mg/dL	280	272	276	270
Total Protein (TP) g/dL	7.3	7.2	7	7.1
TRIG mg/dL	203	198	203	194
Uric Acid (UA) mg/dL	4	4.1	4.1	4.1
VLDL mg/dL	41	40	41	39

3.1.4 PBMN and WBC Irradiations

Isolated PBMN cells or WBCs were seeded at total cell density of 8-10 X10^6 cells, onto culture dishes. Cells were allowed to settle before performing the irradiations. For the alpha-particle exposures, cells were cultured in thin Mylar based plastic dishes (MD) (Chemplex Industries, Palm City, FL, USA), which allowed for penetration of alpha-particles. Irradiations were performed at doses of 0 (control), 0.5, 1.0 or 1.5 Gy using Americium (^{241}Am) electroplated discs with an activity level of 66.0 kBq ± 3% (dose rate of 0.98 ± 0.01 Gy/h, LET of 127.4±0.4 keV/μm). The absorbed dose of alpha-particle radiation to which the cells were exposed was calculated using the GEANT4 v.9.1 Monte Carlo tool-kit (Beaton et al.,2011). Cells destined for X-radiation at doses of 0 Gy, 2 Gy, 5 Gy and 10 Gy were exposed using the X-RAD 320 X-ray irradiation system at a high dose rate of 0.98 ± 0.05 Gy/min, 120 keV (Precision X-ray, Inc., North Branford, CT, USA). Twenty-four hours following irradiation, 50 μl aliquot of cells was removed and assessed for cellular viability using the Trypan Blue viability assay (Bio-rad, Hercules, Ca), 50 μl aliquot was assessed for CBC and thirty minutes post-exposure 100 μl aliquot of cells was analyzed for DNA damage response (gamma-H2AX). Twenty-four hours post-exposure the remainder of the cell population was washed and harvested in 350 μl Qiazol buffer (Qiagen Inc, Mississauga, ON), for transcriptional profiling.

3.1.5 H2AX phosphorylation assay

H2AX phosphorylation was assessed using flow cytometry following a modified protocol by MacPhail et al. (2003). Thirty minutes after exposure, cell suspensions (5×10^5 cells per sample) were washed and fixed with 4% formaldehyde (Fisher Scientific, Hampton, NH, USA) and incubated for 15 min on ice. The cells were then washed and re-suspended in 1 mL cold (−40°C), 70% methanol (Fisher Scientific) and stored at 40 °C overnight or up to two weeks. One mL of cold TBS (triphosphate buffered saline, 0.0154 M Trizma Hydrochloride (Sigma–Aldrich Canada), 0.5 M NaCl (Fisher Scientific), pH 7.4) was then added to each sample, mixed well, centrifuged (8 min, 400 × g, 4 °C) and re-suspended in 1 mL of cold TBS serum triton (TST, 96% TBS, 4% FBS (Sigma–Aldrich Canada), 0.1% Triton X-100 (Sigma–Aldrich Canada)). The samples were incubated on ice for 10 min, centrifuged (5 min, 400 × g, room temperature) and re-suspended in 200 µL of anti-γ-H2AX-FITC (fluorescein isothiocyanate) antibody (Millipore) diluted 1:500 in TST. After a 2 h incubation period on a shaker platform at room temperature, 1 mL of TBS with 2% FBS was added. The samples were then centrifuged (5 min, 400 × g, 4 °C), re-suspended in 400 µL TBS with 2% FBS. Immediately prior to analysis by flow cytometry, 2 µL of 1 mg/mL propidium iodide (PI) was added to each sample.

3.1.6 Flow cytometry analysis

For flow cytometry analysis, data acquisition was set to analyze 2×10^4 cells from the whole cell population as identified by a forward scatter (FSC) vs. side scatter (SSC) dot plot. All debris under the FSC and SSC threshold were excluded from the analysis. The γ-H2AX response was measured by assessing the increased level of intracellular fluorescence characterized in the cells, as determined by the geometric mean of the intensity peak of the anti-γ-H2AX-FITC (channel number) of the γ-H2AX positive cells. The cell cycle distribution was assessed by examining the distribution of the area of the PI signal. All samples were analyzed on a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

3.1.7 RNA extraction

Twenty-four hours following exposure to alpha-particle radiation or negative control conditions, 2 mL of cell culture were transferred to 15 mL Falcon centrifuge tubes (Invitrogen, Canada) and centrifuged at 200 x g for 5 min to pellet the cells. The supernatant were stored for secreted protein analysis, the pelleted cells were resuspended in 350 µL of Buffer RLT containing 1% β-Mercaptoethanol (Qiagen's RNeasy Mini kit; Qiagen Inc, Mississauga, ON) then frozen at -80^{o}C until processed. Frozen lysates were thawed on ice and mixed well by pipetting. The lysate was transferred directly onto a QIAshredder spin column (Qiagen Inc), placed in a 2 mL collection tube and centrifuged for 2 min at ~12,000 g. A volume of 350 µL of 70% ethanol was added. Total RNA was then extracted using the RNeasy Mini kit according to the manufacturer's instructions (Qiagen Inc), with the addition of Qiagen's On-Column RNase-free DNase (Qiagen Inc) to eliminate any remaining DNA contamination. All total RNA sample concentrations and RNA quality were determined using both an Agilent 2100 Bioanalyzer and RNA Nanochips (Agilent Technologies Canada Inc., Mississauga, ON) and spectrophotometrically using an Nanodrop (Fisher Scientific) (OD ratio of A260:A280). All extracted RNA samples were determined to be of good quality (RNA Integrity Number=10) with minimal degradation and stored at -80^{o}C until further analysis. Samples with a RIN value of greater than or equal to 8.0 were deemed to be acceptable for analysis. An input of 200 ng of total RNA was used for whole genome analysis following the Illumina(r) Whole Genome Expression Profiling Assay Guide (11317302 Rev. A). Samples were hybridized on Illumina human-12 v2 RNA BeadChips. BeadChips were imaged and quantified with the Illumina iScan scanner and data was processed with Illumina GenomeStudio v2010.2.

3.1.8 Statistical analysis

Data pre-processing was done within GenomeStudio, where the intensities were averaged per probe/gene. Normalization of dataset was conducted in GeneSpring (version GX 11.5). Intensities were normalized to the 25th percentile. Intensities were log2 transformed and a two tailed T-test were performed. The variance was not assumed to be the same between the groups. Multiple testing using Benjamini & Hochberg (B-H) false discovery correction was applied to the p-values in order to obtain robust responding gene targets (Benjamini et al., 1995).

3.1.9 Quantitative real time-polymerase chain reaction (qRT-PCR) validation

Selected genes identified by microarray analysis as displaying statistical significance and for which primers were validated were further assessed by qRT-PCR. Total RNA (100 ng) isolated from cells were reverse transcribed into complementary DNA using the RT2 First Strand Kit (SABiosciences Corp., Frederick, Maryland, USA). Gene profiling was performed according to the manufacturer instructions using custom RT2-profiler PCR arrays (SABioSciences Corp.). Reactions were prepared in 96-well plates and performed in duplicate in a spectrofluorometric thermal cycler (Biorad iCycler; Hercules, CA). The relative expression of each gene was determined by using the comparative threshold (Ct) method (Livak KJ, Schmittgen, 2001). Analysis of qRT-PCR expression profiles and statistical analysis of data was performed using the super array biosciences web portal for data analysis of their products. (SABiosciences http://www.sabiosciences.com/pcr/arrayanalysis.php).

3.2.0 Pathway analysis

Significantly expressed genes at the high dose of exposure were used for pathway analysis. Gene lists were uploaded into data analysis software (Ingenuity Pathway Analysis (IPA), version 7.5; Ingenuity Systems, CA) (IPA, 2005) and used for core analysis with the following settings: Reference Set = Ingenuity's Knowledge Base (genes only) (IPA, 2009). The same lists were also uploaded as filtered datasets for use in overlaying expression values with the following additional settings: fold-change cut-off =

1.0, P-value cut-off = 0.05, Focus on up- and down-regulated identifiers, resolve duplicates = maximum fold-change, color nodes by fold change. Core comparison analysis was also run to show the differences in top functions and canonical pathways among the different lists. Functional analysis results were obtained after the analysis was complete. The top high-level and corresponding low-level functions were studied to determine the involved genes and whether those genes increase or decrease the specific function, to make conclusions about the mechanisms in flux after exposure to α-particle radiation. Canonical pathway results were obtained after the analysis was complete. Canonical pathway results were customized to display the Benjamini-Hochberg multiple testing correction P-value to assist in omitting false-positive results from the analysis. The threshold used was P-value = 0.05 (5% false positive rate). Canonical pathways that had a P-value of 0.05 or less were further studied to determine the genes that were regulated from these datasets, and how these genes specifically affect the canonical pathway. Networks were used to further corroborate the functional analysis and canonical pathway results and to provide insight into any regulatory mechanisms. Networks were also used to view the molecular connections between the genes of interest to determine if they collectively share common biological functions when working together.

3.2.1 Customized Gene Array Panel

A total of 96 genes were used for the development of a customized 384 well format gene array panel (Figure 1). This panel comprised of genes that were shown by microarray technology to be dose-responsive and also expressed at the medium and high dose. This panel also included several housekeeping genes, control genes that were shown to be unresponsive in the initial microarray screening analysis. SaBiosciences (SABioSciences Corp) designed the primers and provided a 384 well-format platform that was compatible for use on the Roche LightCycler 480 real-time PCR system. A high-throughput PCR platform, comprising the Caliper Zephyr Compact Liquid Handling Station, the Caliper Twister II plate handler, and the Lightcycler 480, was used with custom protocols developed in the Inhalation Toxicology Laboratory of Health Canada (Thomson et al., 2008). This system allowed for rapid screening of 144 samples in a one week time-span. LIMMA (linear models for microarray data) (Smyth 2004 and 2005, Du et al., 2008 and Belstal et al., 2003) was used to identify differentially expressed gene signatures under the various conditions. For each gene in the data a linear model was fit, then an empirical Bayes (EB) method was used to moderate the standard errors for estimating the moderated t-statistics/F-statistics for each gene, this shrinks the standard errors towards a common value. This test is similar to an ANOVA method, except that the residual standard deviations are moderated across genes to ensure more stable inference for each gene. The moderated standard deviations are a compromise between the individual genewise standard deviations and an overall pooled standard deviation. The false discovery rate

ACTA2 1	ACTA2 1	AEN 2	AEN 2	ANKRA2 3	ANKRA2 3	ANXA4 4	ANXA4 4	APOBEC 3H 5	APOBEC 3H 5	ARHGEF 3 6	ARHGEF 3 6	ASCC3 7	ASCC3 7	ASTN2 8	ASTN2 8	BAX 9	BAX 9	BBC3 10	BBC3 10	BTG3 11	BTG3 11	CCDC90B 12	CCDC90B 12
ACTA2 1	ACTA2 1	AEN 2	AEN 2	ANKRA2 3	ANKRA2 3	ANXA4 4	ANXA4 4	APOBEC 3H 5	APOBEC 3H 5	ARHGEF 3 6	ARHGEF 3 6	ASCC3 7	ASCC3 7	ASTN2 8	ASTN2 8	BAX 9	BAX 9	BBC3 10	BBC3 10	BTG3 11	BTG3 11	CCDC90B 12	CCDC90B 12
CCNG1 13	CCNG1 13	CD70 14	CD70 14	CDKN1A 15	CDKN1A 15	CMBL 16	CMBL 16	DCP1B 17	DCP1B 17	DDB2 18	DDB2 18	DRAM1 19	DRAM1 19	E2F7 20	E2F7 20	EDA2R 21	EDA2R 21	EI24 22	EI24 22	FAM127B 23	FAM127B 23	FAM20B 24	FAM20B 24
CCNG1 13	CCNG1 13	CD70 14	CD70 14	CDKN1A 15	CDKN1A 15	CMBL 16	CMBL 16	DCP1B 17	DCP1B 17	DDB2 18	DDB2 18	DRAM1 19	DRAM1 19	E2F7 20	E2F7 20	EDA2R 21	EDA2R 21	EI24 22	EI24 22	FAM127B 23	FAM127B 23	FAM20B 24	FAM20B 24
FAS 25	FAS 25	FBXO22 26	FBXO22 26	FDXR 27	FDXR 27	FHL2 28	FHL2 28	GADD45 A 29	GADD45 A 29	GDF15 30	GDF15 30	GLS2 31	GLS2 31	GNG7 32	GNG7 32	GSS 33	GSS 33	HIST1H4 B 34	HIST1H4 B 34	IER5 35	IER5 35	IGFBP4 36	IGFBP4 36
FAS 25	FAS 25	FBXO22 26	FBXO22 26	FDXR 27	FDXR 27	FHL2 28	FHL2 28	GADD45 A 29	GADD45 A 29	GDF15 30	GDF15 30	GLS2 31	GLS2 31	GNG7 32	GNG7 32	GSS 33	GSS 33	HIST1H4 B 34	HIST1H4 B 34	IER5 35	IER5 35	IGFBP4 36	IGFBP4 36
IL21R 37	IL21R 37	ISCU 38	ISCU 38	ISG20 39	ISG20 39	AEN 40	AEN 40	LAMC3 41	LAMC3 41	LIG1 42	LIG1 42	LY9 43	LY9 43	MAMDC4 44	MAMDC4 44	MAP4K4 45	MAP4K4 45	MDM2 46	MDM2 46	METTL7A 47	METTL7A 47	METTL7A 48	METTL7A 48
IL21R 37	IL21R 37	ISCU 38	ISCU 38	ISG20 39	ISG20 39	AEN 40	AEN 40	LAMC3 41	LAMC3 41	LIG1 42	LIG1 42	LY9 43	LY9 43	MAMDC4 44	MAMDC4 44	MAP4K4 45	MAP4K4 45	MDM2 46	MDM2 46	METTL7A 47	METTL7A 47	METTL7A 48	METTL7A 48
MYC 49	MYC 49	NUDT15 50	NUDT15 50	PCNA 51	PCNA 51	PCNXL2 52	PCNXL2 52	PHLDA3 53	PHLDA3 53	PHPT1 54	PHPT1 54	PLK2 55	PLK2 55	PLK3 56	PLK3 56	POLH 57	POLH 57	PPM1D 58	PPM1D 58	PRKAB1 59	PRKAB1 59	PTP4A1 60	PTP4A1 60
MYC 49	MYC 49	NUDT15 50	NUDT15 50	PCNA 51	PCNA 51	PCNXL2 52	PCNXL2 52	PHLDA3 53	PHLDA3 53	PHPT1 54	PHPT1 54	PLK2 55	PLK2 55	PLK3 56	PLK3 56	POLH 57	POLH 57	PPM1D 58	PPM1D 58	PRKAB1 59	PRKAB1 59	PTP4A1 60	PTP4A1 60
PVT1 61	PVT1 61	RASGRP 2 62	RASGRP 2 62	RetSat 63	RetSat 63	RPS27L 64	RPS27L 64	SAC3D1 65	SAC3D1 65	SESN1 66	SESN1 66	SLC4A11 67	SLC4A11 67	SLC4A11 68	SLC4A11 68	SLC7A6 69	SLC7A6 69	TCF3 70	TCF3 70	TMEM30 A 71	TMEM30 A 71	TMPRSS 7 72	TMPRSS 7 72
PVT1 61	PVT1 61	RASGRP 2 62	RASGRP 2 62	RetSat 63	RetSat 63	RPS27L 64	RPS27L 64	SAC3D1 65	SAC3D1 65	SESN1 66	SESN1 66	SLC4A11 67	SLC4A11 67	SLC4A11 68	SLC4A11 68	SLC7A6 69	SLC7A6 69	TCF3 70	TCF3 70	TMEM30 A 71	TMEM30 A 71	TMPRSS 7 72	TMPRSS 7 72
TNFRSF1 0B 73	TNFRSF1 0B 73	TNFRSF1 0D 74	TNFRSF1 0D 74	TNFSF4 75	TNFSF4 75	TNFSF8 76	TNFSF8 76	TOB1 77	TOB1 77	TP53 78	TP53 78	TP53TG1 79	TP53TG1 79	TP53INP1 80	TP53INP1 80	TP53TG1 81	TP53TG1 81	TRIAP1 82	TRIAP1 82	TRIM22 83	TRIM22 83	TRIM32 84	TRIM32 84
TNFRSF1 0B 73	TNFRSF1 0B 73	TNFRSF1 0D 74	TNFRSF1 0D 74	TNFSF4 75	TNFSF4 75	TNFSF8 76	TNFSF8 76	TOB1 77	TOB1 77	TP53 78	TP53 78	TP53TG1 79	TP53TG1 79	TP53INP1 80	TP53INP1 80	TP53TG1 81	TP53TG1 81	TRIAP1 82	TRIAP1 82	TRIM22 83	TRIM22 83	TRIM32 84	TRIM32 84
UROD 85	UROD 85	VWCE 86	VWCE 86	XPC 87	XPC 87	ZNF337 88	ZNF337 88	ZNF79 89	ZNF79 89	ACTB 90	ACTB 90	GAPDH 91	GAPDH 91	GUSB 92	GUSB 92	B2M 93	B2M 93	HGDC 94	HGDC 94	RTC 95	RTC 95	PPC 96	PPC 96
UROD 85	UROD 85	VWCE 86	VWCE 86	XPC 87	XPC 87	ZNF337 88	ZNF337 88	ZNF79 89	ZNF79 89	ACTB 90	ACTB 90	GAPDH 91	GAPDH 91	GUSB 92	GUSB 92	B2M 93	B2M 93	HGDC 94	HGDC 94	RTC 95	RTC 95	PPC 96	PPC 96

Figure 1: A schematic representation of the 384-well format gene array that was developed for use in the rapid screening and validation of genes that were identified to be alpha particle radiation responsive using microarray technology.

4 Results

4.1 Biological Damage

4.1.1 Complete Blood Count

A representative blood count assessment is provided below (Table 2). Total white blood cell counts were typically in the range of 9-10 x 10^6 cells/ml. The viability of the cells was assessed using the Trypan Blue Viability Assay pre- and post-irradiation. The cells remained viable (above 99%) and no significant changes in blood cell counts were observed post-irradiation relative to un-irradiated cells.

Table 2 : A typical representation of complete blood counts from isolated WBC's obtained from healthy individuals. Values in bracket indicate the percentage of each cell type to the total cell population.

		TotalWhite Blood Cell Count		Post-exposure cell/ml X10^6				
Radiation Type	Dose (Gy)	(cells/ml) X10^6	Viability (%)	Neutrophils	Lymphocytes	Monocytes	Eosonophils	Basophils
alpha	0.0	9.4	99.0	4.07 (43.4)	4.83 (51.5)	0.39 (4.2)	0.07 (0.7)	0.02 (0.2)
alpha	0.5	10.2	99.0	4.96 (48.5)	4.69 (45.8)	0.50 (4.9)	0.06 (0.6)	0.02 (0.2)
alpha	1.0	10.2	99.0	5.04 (49.6)	4.53 (44.6)	0.51 (5.0)	0.07 (0.7)	0.01 (0.1)
alpha	1.5	9.9	99.0	4.98 (50.3)	4.28 (43.2)	0.56 (5.7)	0.06 (0.6)	0.02 (0.2)
x-ray	0.0	10.1	99.0	4.35 (43.0)	5.34 (52.8)	0.35 (3.5)	0.05 (0.5)	0.02 (0.2)
x-ray	2.0	8.9	99.0	3.75 (42.1)	4.80 (53.9)	0.30 (3.4)	0.04 (0.4)	0.02 (0.2)
x-ray	5.0	9.1	99.0	3.75 (41.2)	4.90 (53.8)	0.39 (4.3)	0.05 (0.5)	0.02 (0.2)
x-ray	10.0	9.6	99.0	4.01 (41.9)	5.09 (53.2)	0.42 (4.4)	0.04 (0.4)	0.01 (0.1)

4.1.2 DNA Double Strand Breaks

Thirty minutes post-exposure, cells were assessed for the expression of gamma-H2AX, a marker indicative of DNA double strand breaks. A dose-dependent increase in the γ-H2AX signal was observed following exposure to α-particle radiation as seen by the pronounced shift in the curve and a plot of the geometric mean of this signal as a function of dose (Figure 2). Statistically significant responses were obtained at the medium and high doses tested ($p<0.05$ and $p<0.01$) relative to the non-irradiated control treatment group. A bi-modal shaped curve was observed at the lowest dose of alpha-particle radiation, indicating that only a small population of cells were transversed by alpha-particles. With increasing dose this curve shifted to the right, indicating a greater percentage of double strand breaks. At the highest dose (1.5 Gy) an approximate 3-fold increase in γ-H2AX signal was observed relative to the control sample. As a positive control, isolated lymphocytes were irradiated with X-rays at a high dose rate (1 Gy/min) and a dose range of 2-10 Gy. A plot of this response indicated X-rays to be significantly more damaging as seen by the marked increase in γ-H2AX signal with dose of radiation relative to alpha particle treated cells. Alpha-particle irradiated cells showed less damage relative to the X-irradiated cells, potentially due to the activation of repair mechanisms that normally occur at slower dose-rates of exposure.

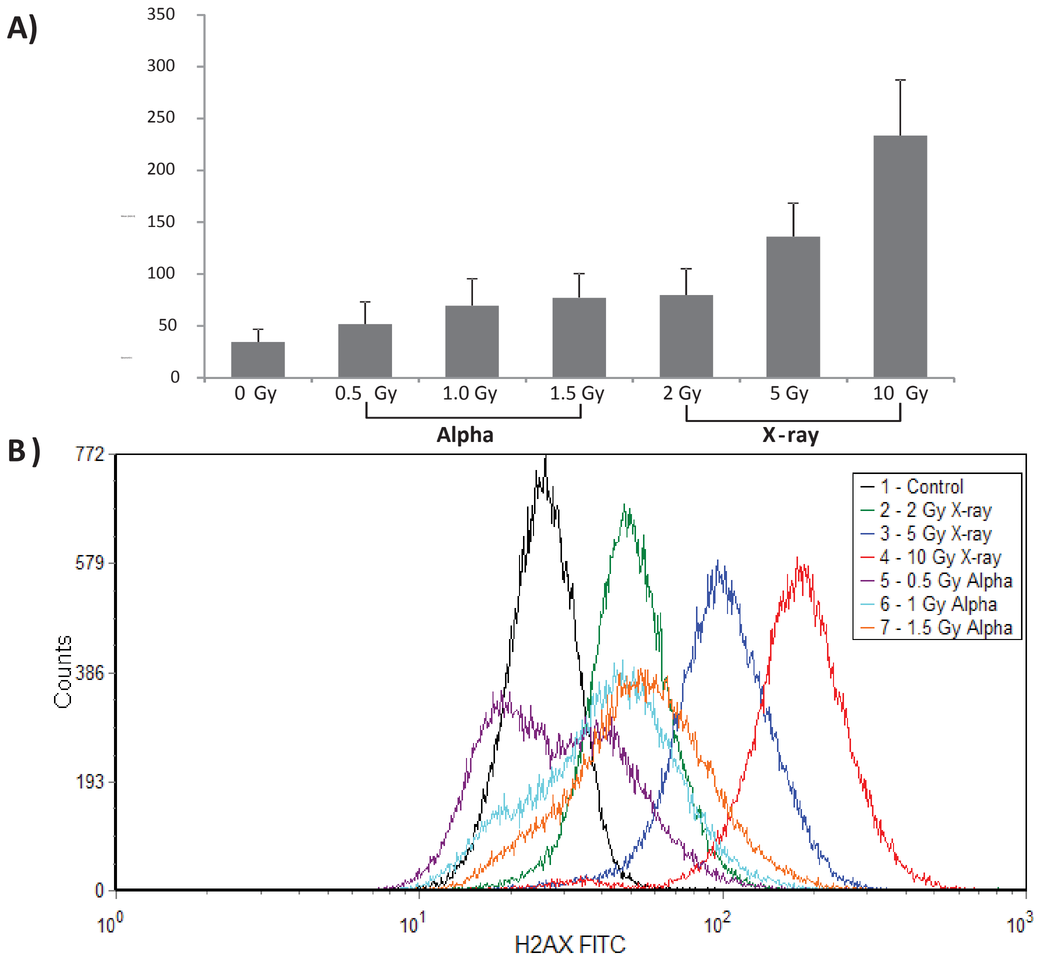


Figure 2: A) A plot of geometric mean (indicative of γ-H2AX expression) for each of the doses and radiation types. Data is presented as means ± SEM with n= 12 biological replicates. B) A representative flow cytometric histogram overlay of γ-H2AX expression in WBC cells at various doses of α-particle and X-ray exposure measured 30 min post-exposure.

4.2 Gene Expression Assessment and Validation

4.2.1 Genomic Profiling

Genomic profiling using Illumina Bead array platform was performed on RNA extracted from isolated PBMN cells 24 h post-exposure. Stringent statistical methods were employed including Benjamini Hochberg (B-H) false discovery correction in order to mine for reliable genes. All differentially expressed genes were filtered on flagged spots that were poor quality, and a B-H p-value <0.05. A summary of the gene responses at each of the doses is provided in Table 3 Overall transcripts were shown be upregulated in the presence of the radiation injury. A Venn diagram was constructed to provide a quantitative representation of the similarities and differences in expression profiles at each of the doses (Figure 3). Thirty one genes were shown to be expressed at all three doses with expression levels ranging from 2-10 fold. Forty four genes were expressed at both the medium and high dose. The range in expression levels of these genes is summarized as a heat map which delineates the high expressors from the low expressors (Figure 4). These genes alongside the 31 genes expressed at all three doses were further validated using qRT-PCR.

Table 3 : A summary of the number of differentially expressed transcripts that were obtained from exposure of isolated PBMC to alpha particle radiation at each of the radiation doses. The number in brackets indicate the percentage of transcripts that were expressed in each of the categories from the total number of responding genes at each dose.

	0.5 Gy	1.0 Gy	1.5 Gy
Number of Transcripts	**33**	**77**	**154**
Common Amongst Doses *(%)*	**31** (94)	**31** *(40)*	**31** *(20)*
Exclusive *(%)*	2 (6)	2 *(3)*	79 *(51)*
Up Regulated *(%)*	**31** (94)	**74** *(96)*	**135** *(88)*
Common Amongst Doses *(%)*	31 (100)	31 *(46)*	31 *(23)*
Exclusive *(%)*	0 (0)	1 *(1)*	62 *(46)*
Down Regulated *(%)*	**2** (6)	**3** *(4)*	**19** *(12)*
Common Amongst All Doses *(%)*	0 (0)	0 *(0)*	0 *(0)*
Exclusive *(%)*	2 (100)	1 *(33)*	17 *(89)*

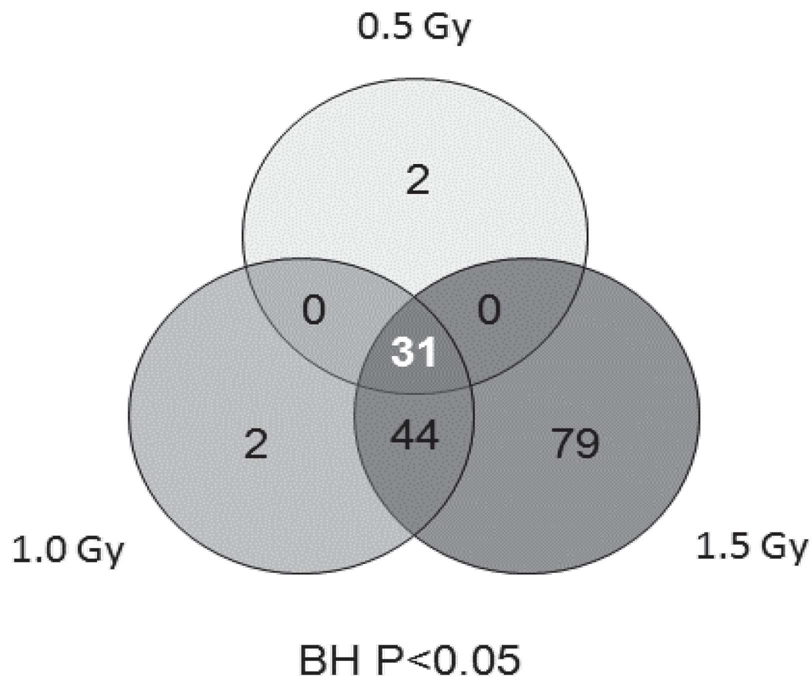
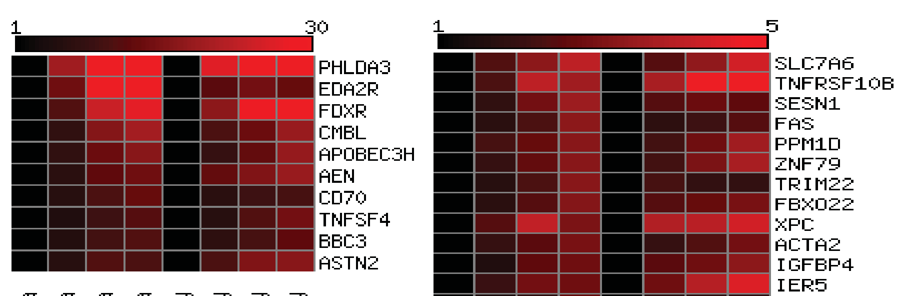


Figure 3: A Venn diagram depicting the exclusive and common genes which were shown to be significantly modulated via microarray in peripheral blood mononuclear cells (PBMCs) following various doses of alpha-particle radiation. Based on an n=12 human donors.



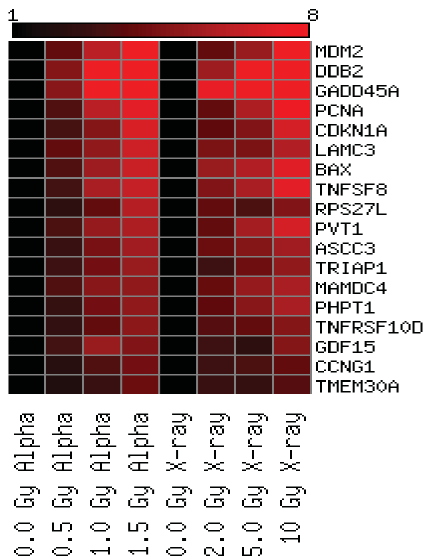


Figure 4: Heat map depicting the microarray fold change expression values of the genes which were found to be statistically significant at the medium and high doses and at all three doses of radiation exposure using microarray technology. Red colouring signifies up-regulation based on an n=12 biological replicates. The heat map is divided into three profiles based on the expression levels (high, medium, and low).

4.2.2 qRT-PCR Validation

The forty transcripts observed to be expressed at the medium and high dose and the 31 genes expressed at all three doses were further validated using qRT-PCR (Table 4). Of these genes, five genes (I*SG20L1*, *AEN*, *mir1204*, *TP531AP1*, *LOC90120*, *LOC387882*) could not be further validated due to the unavailability of efficient primers for cDNA amplification. A comparison of the responses using the two technologies showed a similar trend in expression levels. As shown in Table 4, all 70 genes that exhibited a significant response at the three doses using microarray analysis were also observed to exhibit a similar trend using qRT-PCR. Genes that were expressed at both the medium and high dose also displayed similar fold expression changes between the two methodologies however, for a few selected genes, the significance level did not always correspond. Approximately 20 of the total validated targets were shown to be non-significant using qRT-PCR, potentially due to the low expression levels of these transcripts.

Table 4: Validation of gene responses. A comparison of the gene expression fold change (FC) responses obtained using microarray technology (MA) and qRT-PCR (qPCR) at the three doses . Transcripts that were shown to differentially expressed at the medium and high dose and at all three dose were validated using qPCR . PV indicates the p-value obtained for n-12 individuals using Student T-test. The table is divided into two sections. The first section is all genes that were shown to be significant using MA technology at all three doses. The second section are those genes that were shown to be significant using MA technology at the medium and high dose.

Technology	qPCR		MA		qPCR		MA		qPCR		MA	
Symbol	FC	PV	FC	PV	FC	PV	FC	PV	FC	PV	FC	PV
All Doses												
TRIAP1	2.15	0.00	1.81	0.00	3.32	0.00	2.14	0.00	3.78	0.00	2.57	0.00
GADD45A	2.94	0.00	2.27	0.01	4.43	0.00	2.77	0.00	4.70	0.00	3.38	0.00
RPS27L	2.01	0.00	1.67	0.01	2.59	0.00	1.90	0.00	3.57	0.00	2.04	0.00
MAP4K4	1.51	0.01	1.40	0.05	1.79	0.00	1.54	0.00	1.90	0.00	1.69	0.00
TNFRSF10D	2.15	0.01	1.81	0.01	2.94	0.00	2.08	0.00	3.08	0.00	2.39	0.00
ASTN2	6.18	0.01	2.07	0.01	8.02	0.00	2.67	0.00	8.98	0.00	3.15	0.00
TNFRSF10B	2.28	0.01	2.02	0.01	3.06	0.00	2.32	0.00	3.13	0.00	2.72	0.00
TMEM30A	1.92	0.01	1.72	0.01	2.67	0.00	2.13	0.00	2.76	0.00	2.45	0.00
TP53INP1	1.56	0.01	1.53	0.04	1.83	0.00	1.81	0.00	1.88	0.00	2.02	0.00
BAX	2.42	0.01	2.31	0.00	3.17	0.00	2.88	0.00	3.89	0.00	3.23	0.00
FAS	1.89	0.01	1.79	0.00	2.61	0.00	2.06	0.00	2.94	0.00	2.23	0.00
DDB2	2.96	0.01	3.56	0.00	5.15	0.00	4.41	0.00	5.26	0.00	5.49	0.00
AEN	4.24	0.01	2.16	0.01	7.09	0.00	2.80	0.00	7.46	0.00	3.14	0.00
GLS2	1.94	0.01	1.87	0.01	2.30	0.00	2.37	0.00	2.46	0.00	2.62	0.00
CMBL	3.99	0.01	2.52	0.01	6.79	0.00	2.90	0.00	7.41	0.00	3.57	0.00
PHPT1	2.28	0.02	2.23	0.00	3.50	0.00	2.57	0.00	4.17	0.00	3.03	0.00
PCNA	2.49	0.02	2.02	0.00	3.64	0.00	2.44	0.00	4.09	0.00	2.88	0.00
ASCC3	2.14	0.03	1.84	0.00	3.18	0.00	2.22	0.00	3.28	0.00	2.37	0.00
PPM1D	1.58	0.05	1.63	0.01	2.14	0.00	1.81	0.00	2.17	0.00	2.11	0.00
XPC	1.62	0.05	1.81	0.01	2.20	0.00	2.12	0.00	2.00	0.01	2.31	0.00
IER5	2.09	0.05	1.82	0.01	2.71	0.00	2.10	0.00	3.09	0.00	2.41	0.00
TNFSF4	2.91	0.06	2.93	0.00	4.98	0.00	3.69	0.00	5.34	0.00	4.67	0.00
SESN1	1.40	0.06	1.90	0.00	2.25	0.00	2.14	0.00	1.98	0.01	2.44	0.00
FBXO22	1.40	0.08	1.39	0.03	1.97	0.01	1.56	0.00	2.17	0.00	1.65	0.00
PHLDA3	9.91	0.09	3.18	0.00	22.89	0.00	3.96	0.00	28.82	0.00	4.89	0.00
ACTA2	2.12	0.09	2.54	0.01	2.72	0.01	3.07	0.00	2.74	0.01	3.60	0.00
CCNG1	0.91	0.10	1.80	0.00	2.49	0.00	1.97	0.00	2.56	0.00	2.14	0.00
MAMDC4	2.54	0.14	2.06	0.04	3.18	0.02	2.82	0.00	3.52	0.01	3.23	0.00
APOBEC3H	2.38	0.55	2.52	0.04	3.68	0.08	3.20	0.00	4.49	0.02	3.91	0.00

Radiation Type	0.5 Gy Alpha				1.0 Gy Alpha				1.5 Gy Alpha			
Technology	qPCR		MA		qPCR		MA		qPCR		MA	
Symbol	FC	PV	FC	PV	FC	PV	FC	PV	FC	PV	FC	PV
Med/High Doses												
SAC3D1	1.47	0.01	1.26	0.24	1.74	0.00	1.29	0.04	1.91	0.01	1.33	0.01
PVT1	2.02	0.01	1.42	1.00	2.76	0.00	1.68	0.02	3.45	0.00	1.85	0.00
TP53TG1	1.42	0.01	1.20	1.00	1.60	0.00	1.39	0.03	1.92	0.01	1.45	0.01
TNFSF8	2.40	0.01	1.92	0.08	4.01	0.00	2.33	0.00	3.80	0.00	2.61	0.00
TMPRSS7	4.04	0.01	1.24	1.00	4.85	0.01	1.53	0.00	6.74	0.00	1.52	0.00
ZNF79	1.74	0.02	1.39	0.47	2.07	0.00	1.74	0.00	2.34	0.00	1.95	0.00
SLC7A6	1.64	0.03	1.62	0.15	1.98	0.01	1.89	0.00	2.22	0.00	2.15	0.00
CDKN1A	2.41	0.03	2.30	0.11	3.87	0.00	2.94	0.00	5.05	0.00	3.58	0.00
LIG1	1.66	0.04	1.27	1.00	1.77	0.01	1.53	0.01	1.88	0.01	1.57	0.00
MDM2	2.93	0.04	1.24	0.31	4.44	0.00	1.30	0.02	5.17	0.00	1.45	0.00
FDXR	6.46	0.06	1.47	0.85	13.07	0.00	1.76	0.01	14.43	0.00	1.88	0.00
DCP1B	1.68	0.06	1.17	1.00	2.00	0.00	1.45	0.01	2.33	0.00	1.51	0.00
BBC3	4.10	0.06	1.40	0.66	6.75	0.00	1.65	0.01	7.25	0.00	1.83	0.00
ISG20	0.75	0.06	-1.44	1.00	0.46	0.00	-1.92	0.00	0.46	0.00	-2.30	0.00
SESN1	1.40	0.06	1.29	1.00	2.25	0.00	1.57	0.03	1.98	0.01	1.81	0.00
FAM20B	1.49	0.07	1.08	1.00	1.45	0.01	1.25	0.03	1.55	0.01	1.35	0.00
DRAM1	1.41	0.07	1.32	1.00	1.70	0.00	1.55	0.03	1.91	0.00	1.66	0.00
CCDC90B	2.18	0.07	1.20	0.64	1.54	0.07	1.29	0.02	2.89	0.00	1.35	0.00
POLH	1.72	0.08	1.39	0.24	2.37	0.00	1.60	0.00	2.10	0.01	1.92	0.00
GSS	1.19	0.08	1.15	1.00	1.34	0.01	1.29	0.03	1.60	0.00	1.34	0.00
PRKAB1	1.40	0.08	1.29	0.59	1.62	0.01	1.49	0.00	1.85	0.00	1.60	0.00
ARHGEF3	1.37	0.09	1.32	0.59	1.74	0.01	1.51	0.00	1.68	0.02	1.55	0.00
ZNF337	1.23	0.11	1.34	0.25	1.53	0.02	1.44	0.01	1.61	0.02	1.52	0.00
HIST1H4B	1.34	0.11	1.83	0.75	1.62	0.02	2.21	0.03	1.79	0.01	2.59	0.00
LAMC3	2.76	0.12	1.54	1.00	3.63	0.00	1.93	0.02	4.50	0.00	2.13	0.00
GDF15	1.84	0.14	1.49	1.00	2.40	0.01	1.98	0.03	2.96	0.05	2.26	0.00
ISCU	1.15	0.15	1.18	0.98	1.29	0.01	1.24	0.05	1.47	0.00	1.26	0.01
TRIM22	1.28	0.15	1.69	0.25	1.96	0.02	2.06	0.00	1.63	0.02	2.16	0.00
BTG3	1.11	0.23	1.30	0.21	1.69	0.00	1.41	0.00	1.70	0.01	1.54	0.00
NUDT15	1.22	0.23	1.21	0.24	1.48	0.02	1.31	0.00	1.47	0.03	1.38	0.00
RetSat	1.25	0.27	1.21	1.00	1.16	0.56	1.38	0.03	1.19	0.29	1.45	0.00
TRIM32	1.32	0.27	1.25	0.45	1.10	0.15	1.34	0.02	1.58	0.04	1.37	0.00
FHL2	1.74	0.30	1.50	0.44	2.47	0.02	1.82	0.00	2.65	0.01	2.20	0.00
FAM127B	1.14	0.30	1.21	0.54	1.53	0.01	1.31	0.00	1.58	0.05	1.38	0.00
PCNXL2	1.23	0.37	1.19	1.00	1.18	0.52	1.33	0.01	1.34	0.15	1.40	0.00
E2F7	2.23	0.37	1.33	1.00	3.19	0.39	1.49	0.05	4.08	0.43	1.72	0.00
TOB1	1.02	0.40	1.23	0.69	1.40	0.02	1.38	0.00	1.40	0.03	1.47	0.00
ANKRA2	1.07	0.44	1.39	0.27	1.63	0.01	1.48	0.02	1.60	0.02	1.60	0.00
CD70	2.45	0.55	2.04	0.75	4.44	0.04	2.50	0.04	5.05	0.03	3.01	0.00
EDA2R	8.96	0.61	1.31	1.00	19.58	0.87	1.57	0.02	31.64	0.29	1.67	0.00
IGFBP4	1.19	0.68	1.19	1.00	1.61	0.06	1.45	0.01	1.63	0.05	1.52	0.00

4.2.3 Pathway Analysis

Statistically significant genes expressed at the 1.5 Gy alpha-aprticle dose were inputted into the IPA analysis tool to determine interconnectivity of the genes and associated canonical pathways (Figure 5). The data showed similar responding pattern of effects to previous work using human keratinocytes (Chauhan et al., 2012). The top networks associated with this gene set included apoptosis, cell-to-cell signaling interaction, cell death and delay in cell cycle progression. The top bio-functions in relation to diseases and disorders were apoptosis of lung cancer, respiratory disease and inflammatory response. Molecular and cellular functions of these genes were in relation to lipid metabolism, molecular transport and cellular movement. In terms of physiological system development and function the top bio-functions of the genes was in relation to haematological system development and function, immune cell trafficking, lymphoid tissue structure and development. The top canonical pathways associated with these genes were *p53* signaling, *GADD45* signaling and molecular mechanisms of cancer. Several high expressing genes associated with these pathways included *CCNG1*, *PCNA*, *PMAIP1*,*TP53INP1*, *GADD45A*, *BBC3*, *CDKN1A*, *TNFRSF10B*, *MDM2*, *BAX*, *FAS*, *LRP5*, *GS5*, *RPMID*, *PRKAB1*, *RPS276*, *SPATA18*, *SRA1*, *RNU6-1*, *LDA3, PHLDA3* and *DRAM1*. All were upregulated in expression with the exception of *PACS1N1* which was downregulated by ~2 fold following α-particle irradiation.

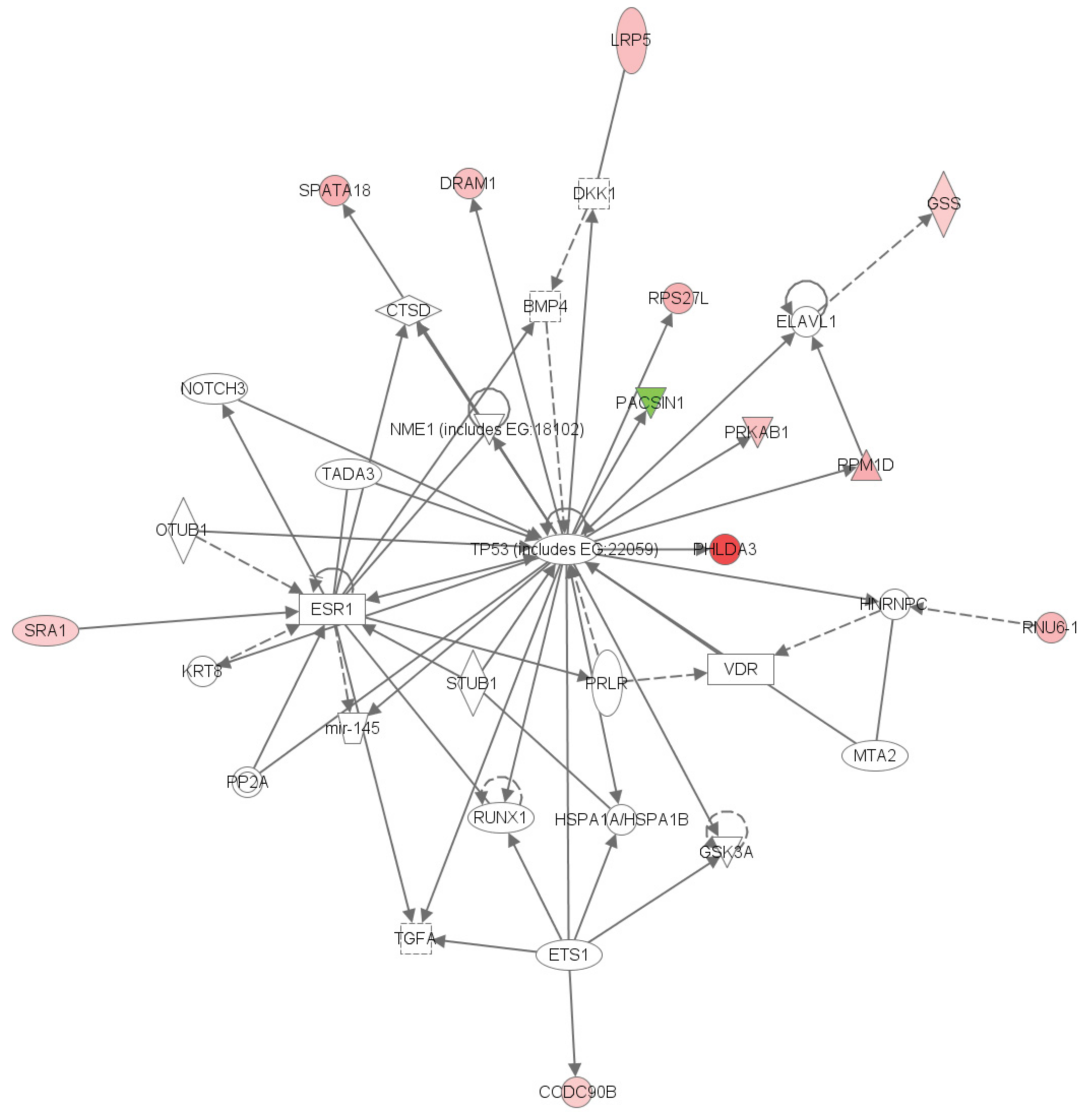


Figure 5 : Network assembled from the genes responsive at the high dose and clustered around TP-53 signalling. Red represents upregulated genes and green represents downregulated genes.

4.3 Custom PCR array Validation

4.3.1 Custom qRT-PCR panel

The alpha-particle responsive genes were employed for the development of a customized gene array panel that included controls and housekeeping genes. This panel was used to verify the validity, specificity and overall integrity of alpha-particle responsive genes in a new population of cells. Targeted cells used for validation were the entire white blood cell population. WBC's were isolated from 12 healthy individuals and exposed to alpha-particles and X-rays. Twenty-four hours post-exposure, RNA was extracted and converted to cDNA for assessment of gene expression modulations using qRT-PCR. All dose-responsive alpha-particle responsive genes were observed to be significantly expressed at the three doses in the total WBC population. Among the 41 genes observed at the medium and high dose, 15 were shown to be un-responsive at the medium dose, however the majority of these were observed at high dose of radiation (Table 5). Stringent statistical analyses were employed to identify these robust responding genes. However, qRT-PCR data does not normally correct for false-discovery using B-H corrections. When the data was assessed using non-stringent statistical methods the results were more comparable to the microarray dataset (data not shown). Box plots of the responding genes allowed for a comparative assessment of the two radiation types and for an assessment of the range in inter-individual variability between transcripts (Figures 6-9). Of particular note is the lack of significant variability among individuals in the control treatment groups, implying a stable baseline expression of these genes in un-irradiated cells. Radiation exposure significantly modulated all genes shown to be alpha-responsive. Although all genes shown to be expressed in the alpha-particle treated cells were also observed in the X-irradiated cells, the level of expression was not similar. This trend would be more prominent if the doses and dose-rates were matched between the two radiation types, irrespective of the inherent differences between the radiations' linear energy transfer qualities.

Table 5: A customized gene array panel was designed using the differentially expressed genes shown to be alpha-particle responsive using microarray technology. The dose-responsive genes alongside the genes that were expressed at the medium and high dose were targeted for further validation using qRT-PCR. WBCs were isolated from 12 individuals and ex-vivo irradiated with alpha particles and X-rays. RNA was extracted 24h post-exposure and assessed for differential response using qRT-PCR using the customized gene array panel. FC=fold change; PV=p-value.

Radiation Type	Alpha						X-Ray					
Dose (Gy)	0.5		1.0		1.5		2.0		5.0		10.0	
GeneID	FC	PV	FC	PV	FC	PV	FC	PV	FC	PV	FC	PV
All Doses												
DDB2	4.65	0.00	8.45	0.00	8.76	0.00	5.37	0.00	8.59	0.00	9.12	0.00
PCNA	3.30	0.00	6.05	0.00	7.27	0.00	3.80	0.00	5.76	0.00	7.47	0.00
AEN	6.02	0.01	12.22	0.00	13.86	0.00	12.67	0.00	15.88	0.00	18.58	0.00
AEN	6.02	0.01	12.22	0.00	13.86	0.00	12.67	0.00	15.88	0.00	18.58	0.00
TNFSF4	4.71	0.03	9.04	0.00	11.01	0.00	5.56	0.00	10.64	0.00	14.46	0.00
PHPT1	2.65	0.03	4.22	0.00	4.96	0.00	3.68	0.00	4.73	0.00	5.70	0.00
TNFRSF10B	2.29	0.03	3.94	0.00	3.52	0.00	3.66	0.00	5.27	0.00	5.47	0.00
MAP4K4	1.90	0.05	2.29	0.00	2.49	0.00	2.03	0.00	2.28	0.00	2.50	0.00
GLS2	2.00	0.06	2.45	0.00	2.62	0.00	2.66	0.00	3.73	0.00	4.37	0.00

ACTA2	1.99	0.07	2.48	0.00	2.90	0.00	1.95	0.01	2.35	0.00	2.82	0.00
TRIAP1	2.88	0.07	4.27	0.00	5.18	0.00	2.87	0.00	4.11	0.00	5.03	0.00
IER5	2.02	0.09	2.82	0.00	2.79	0.00	2.75	0.00	3.85	0.00	4.47	0.00
APOBEC3H	7.03	0.10	13.35	0.00	16.63	0.00	7.82	0.00	12.59	0.00	17.99	0.00
TNFRSF10D	2.64	0.10	3.83	0.00	4.91	0.00	3.49	0.00	3.81	0.00	4.66	0.00
XPC	2.43	0.13	4.00	0.00	2.99	0.00	3.76	0.00	3.89	0.00	4.28	0.00
PPM1D	2.21	0.13	2.65	0.02	3.18	0.00	2.17	0.00	2.81	0.00	3.55	0.00
BAX	3.35	0.15	5.35	0.00	6.56	0.00	5.21	0.00	5.83	0.00	7.19	0.00
PHLDA3	19.38	0.23	50.31	0.00	46.15	0.00	26.63	0.00	37.99	0.00	47.11	0.00
ASTN2	5.85	0.24	10.73	0.01	10.09	0.01	10.41	0.00	15.76	0.00	16.68	0.00
GADD45A	4.76	0.30	8.33	0.01	8.07	0.00	7.35	0.00	10.87	0.00	14.02	0.00
MAMDC4	3.32	0.36	4.77	0.03	4.98	0.01	3.86	0.00	5.09	0.00	5.68	0.00
CMBL	7.34	0.43	16.13	0.01	19.65	0.00	10.40	0.00	13.33	0.00	18.53	0.00
ASCC3	2.82	0.53	4.35	0.08	5.48	0.01	4.06	0.00	4.63	0.02	5.45	0.00
CCNG1	2.12	0.55	3.35	0.05	4.22	0.00	2.88	0.02	3.23	0.03	3.80	0.00
FBXO22	1.70	0.66	2.41	0.16	3.10	0.02	2.39	0.01	2.63	0.06	2.93	0.01
TP53INP1	1.46	0.66	1.80	0.28	2.20	0.04	1.73	0.05	1.74	0.33	2.10	0.06
FAS	1.72	0.72	2.28	0.30	3.19	0.02	1.80	0.07	2.07	0.46	2.41	0.20
RPS27L	2.34	0.99	3.76	0.89	5.96	0.61	3.83	0.05	3.23	0.90	4.57	0.67
TMEM30A	1.91	0.99	2.82	0.87	4.04	0.56	2.82	0.06	2.70	0.90	3.42	0.67

Radiation Type			**Alpha**						**X-Ray**			
Dose (Gy)	**0.5**		**1.0**		**1.5**		**2.0**		**5.0**		**10.0**	
GeneID	**FC**	**PV**	**FC**	**PV**	**FC**	**PV**	**FC**	**PV**	**FC**	**PV**	**FC**	**PV**
Med/High dose												
BBC3	5.15	0.03	9.60	0.00	10.83	0.00	6.80	0.00	9.67	0.00	11.92	0.00
TNFSF8	2.98	0.03	5.64	0.00	6.38	0.00	4.57	0.00	5.65	0.00	7.34	0.00
PVT1	3.20	0.04	5.09	0.00	5.76	0.00	3.76	0.00	5.58	0.00	6.89	0.00
FDXR	10.88	0.05	24.04	0.00	27.24	0.00	17.01	0.00	27.84	0.00	37.91	0.00
TRIM32	1.80	0.05	1.96	0.01	2.01	0.01	1.71	0.01	2.11	0.00	2.48	0.00
ANKRA2	1.54	0.05	2.07	0.00	2.15	0.00	1.73	0.00	2.01	0.00	2.12	0.00
GSS	1.44	0.05	1.67	0.00	1.69	0.00	1.44	0.01	1.72	0.00	2.01	0.00
LIG1	1.89	0.05	2.61	0.00	2.44	0.00	2.27	0.00	2.83	0.00	2.98	0.00
HIST1H4B	1.79	0.07	2.06	0.00	1.96	0.01	1.67	0.04	2.09	0.00	2.59	0.00
ARHGEF3	1.62	0.07	2.03	0.00	2.20	0.00	1.98	0.00	2.47	0.00	2.83	0.00
SLC7A6	2.37	0.09	3.19	0.00	3.94	0.00	2.42	0.00	3.25	0.00	4.28	0.00
CD70	5.76	0.13	10.08	0.00	12.92	0.00	6.14	0.00	9.06	0.00	9.89	0.00
ZNF79	1.96	0.13	2.58	0.00	3.17	0.00	2.18	0.00	2.95	0.00	3.68	0.00
TOB1	1.39	0.13	1.61	0.00	1.74	0.00	1.26	0.13	1.63	0.00	1.78	0.00
ZNF337	1.42	0.37	1.88	0.00	2.08	0.00	1.66	0.01	1.92	0.00	2.30	0.00
PRKAB1	1.67	0.38	2.06	0.05	2.32	0.02	1.81	0.01	2.06	0.01	2.38	0.00
BTG3	1.58	0.38	1.87	0.04	2.15	0.01	1.66	0.02	1.94	0.01	2.06	0.00
TP53	-1.19	0.38	-1.42	0.02	-1.45	0.01	-1.33	0.07	-1.40	0.01	-1.53	0.00
POLH	1.74	0.42	2.34	0.07	2.16	0.13	2.01	0.02	2.44	0.02	2.92	0.00
MDM2	3.83	0.43	6.10	0.05	8.91	0.00	3.76	0.01	5.24	0.01	8.33	0.00
DCP1B	1.84	0.46	2.59	0.04	2.37	0.07	2.47	0.00	2.98	0.00	3.39	0.00
FAM20B	1.41	0.53	1.54	0.23	1.60	0.17	1.50	0.02	1.73	0.01	1.91	0.00
SESN1	1.83	0.55	2.86	0.04	3.45	0.00	2.41	0.02	2.72	0.04	2.54	0.02
PCNXL2	1.26	0.55	1.44	0.09	1.45	0.11	1.23	0.21	1.51	0.02	1.76	0.00

ISG20	-1.18	0.58	-1.53	0.11	-1.36	0.28	-1.55	0.49	-1.67	0.11	-1.61	0.18
CDKN1A	3.12	0.62	4.70	0.20	6.97	0.02	3.67	0.01	4.52	0.13	6.84	0.00
LAMC3	3.76	0.66	4.94	0.47	6.68	0.21	4.42	0.02	4.42	0.33	5.89	0.12
DRAM1	1.54	0.66	2.00	0.19	2.62	0.02	1.56	0.12	1.92	0.25	2.17	0.08
RetSat	1.27	0.66	1.42	0.28	1.19	0.35	1.82	0.02	2.09	0.01	2.35	0.00
ISCU	1.25	0.66	1.43	0.22	1.86	0.01	1.45	0.03	1.53	0.11	1.62	0.02
EDA2R	13.97	0.73	30.23	0.36	39.14	0.15	11.63	0.02	14.28	0.27	13.00	0.20
NUDT15	1.48	0.74	1.85	0.23	2.66	0.03	1.73	0.03	1.84	0.25	1.91	0.14
FHL2	1.66	0.74	2.77	0.16	2.56	0.20	1.69	0.05	2.23	0.13	2.84	0.01
CCDC90B	1.42	0.88	1.68	0.53	2.28	0.04	1.56	0.22	1.57	0.83	1.82	0.42
IGFBP4	1.51	0.92	2.54	0.55	2.81	0.40	2.45	0.02	2.77	0.37	3.23	0.15
SAC3D1	1.46	0.94	1.69	0.77	2.01	0.56	2.04	0.04	2.12	0.51	2.85	0.17
E2F7	-1.98	0.94	-1.26	0.87	1.26	0.92	-1.00	0.31	1.56	0.51	2.61	0.50
FAM127B	1.57	0.98	1.56	0.90	2.18	0.52	1.94	0.06	1.74	0.90	1.96	0.76
TMPRSS7	1.66	0.99	1.55	0.73	2.56	0.76	4.16	0.13	5.92	0.34	8.32	0.05
GDF15	2.98	0.99	5.21	0.87	4.54	0.78	2.90	0.05	2.37	0.94	4.61	0.62
TRIM22	1.67	0.99	2.27	0.89	3.15	0.56	2.20	0.06	1.91	0.96	1.89	0.85
TP53TG1	1.37	0.99	1.62	0.90	2.32	0.49	1.73	0.07	1.53	0.97	1.95	0.77
TP53TG1	1.37	0.99	1.62	0.90	2.32	0.63	1.73	0.08	1.53	0.97	1.95	0.83
Radiation Type	**Alpha**						**X-Ray**					
Dose	**0.5**		**1.0**		**1.5**		**2.0**		**5.0**		**10.0**	
GeneID	**FC**	**PV**	**FC**	**PV**	**FC**	**PV**	**FC**	**PV**	**FC**	**PV**	**FC**	**PV**
Control												
GNG7	-1.16	0.99	-1.30	0.55	-1.44	0.49	1.27	0.10	-1.07	0.87	-1.19	0.63

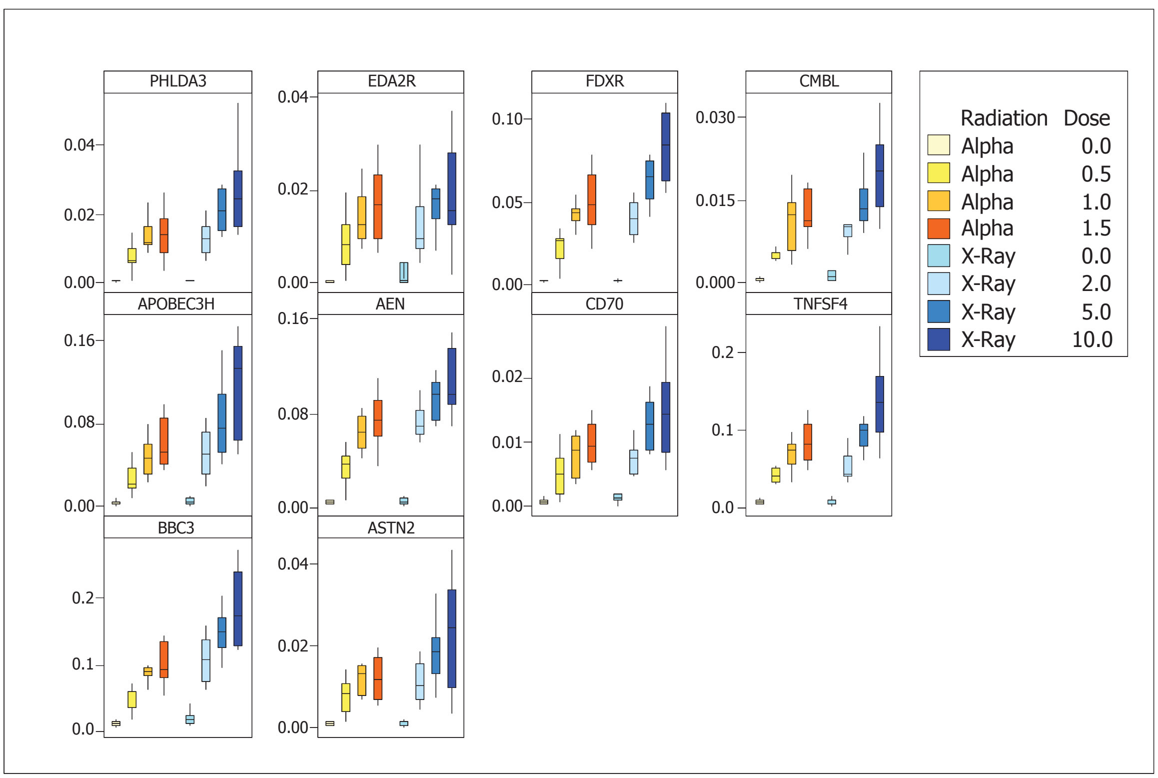


Figure 6: Box-plots of differentially expressed genes assessed using qRT-PCR of cDNA isolated from 12 individuals treated with alpha-particle radiation and X-rays. The ***high*** *responding genes that corresponded to the heat-map are plotted. The central line represents the median of the data and the box edges represent the upper (75th) and lower (25th) percentile. Whiskers denote the highest and lowest values from the data set within the upper and lower limits. Limits are defined as 1.5*50 percentile spread.*

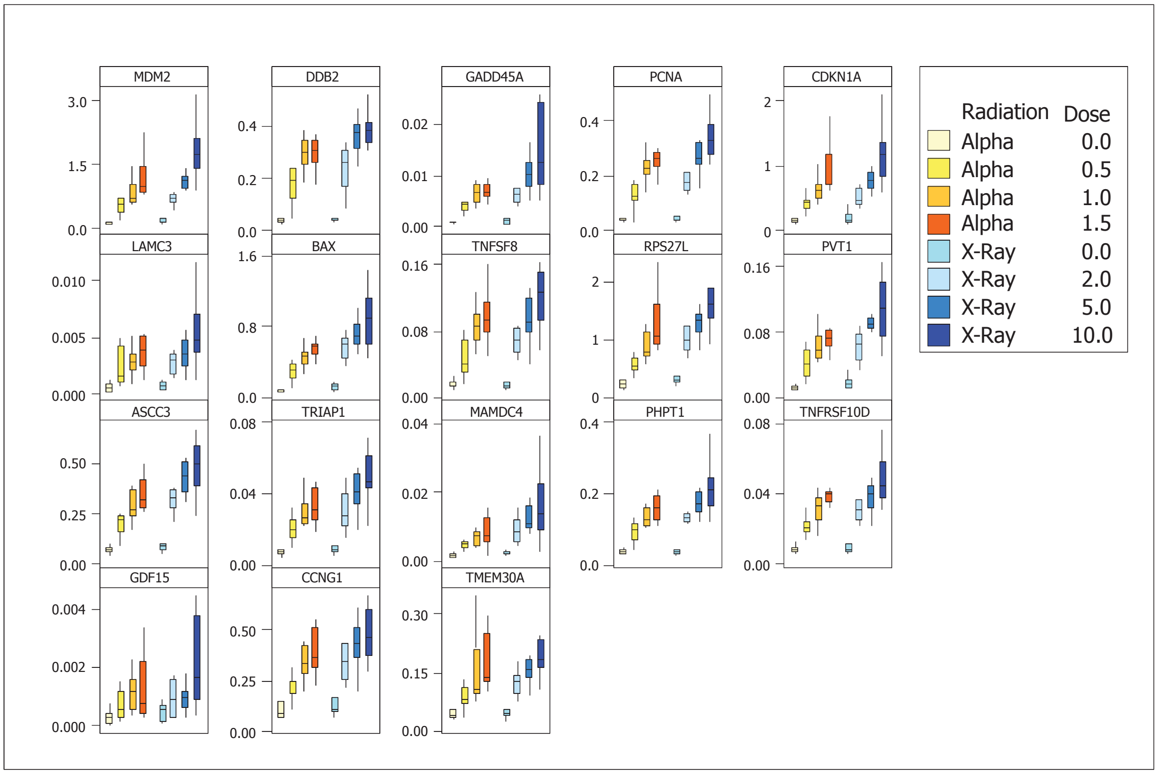


Figure 7: Box-plots of differentially expressed genes assessed using qRT-PCR of cDNA isolated from 12 individuals treated with alpha particle radiation and X-rays. The ***medium*** *responding genes that corresponded to the heat-map are plotted. The central line represents the median of the data and the box edges represent the upper (75th) and lower (25th) percentile. Whiskers denote the highest and lowest values from the data set within the upper and lower limits. Limits are defined as 1.5*50 percentile spread.*

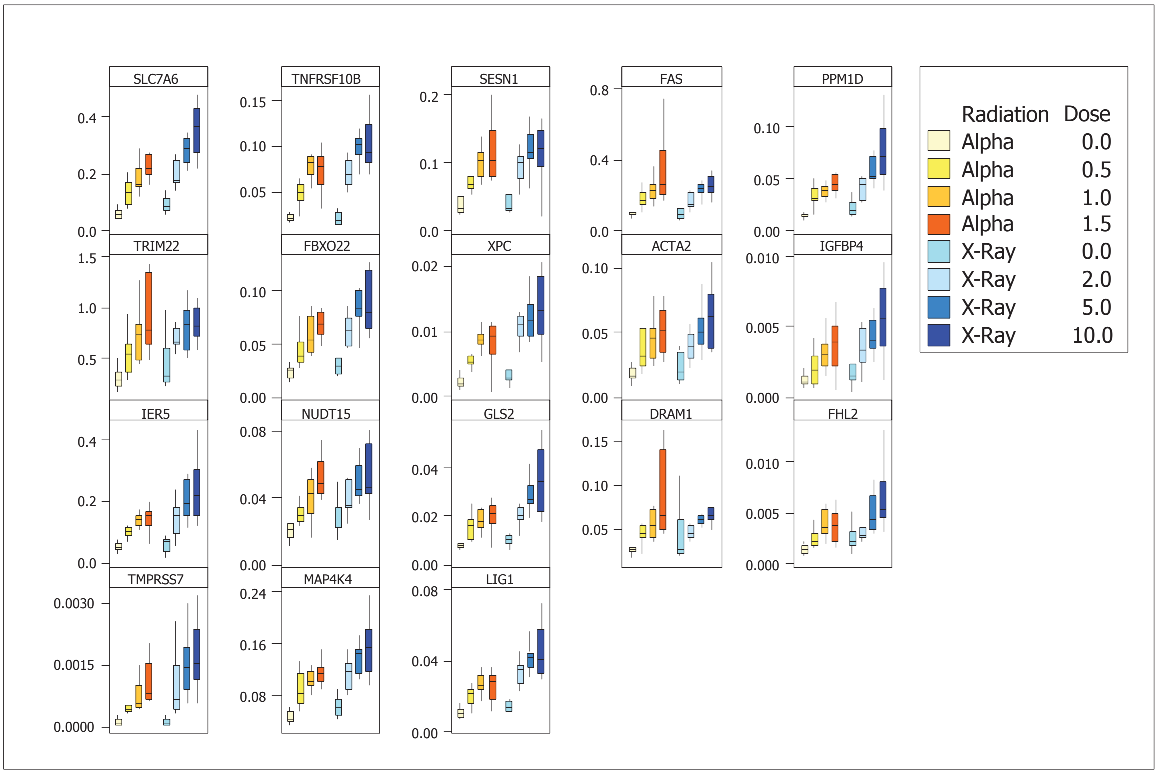


Figure 8: Box-plots of differentially expressed genes assessed using qRT-PCR of cDNA isolated from 12 individuals treated with alpha particle radiation and X-rays. The ***low*** *responding genes that corresponded to the heat-map are plotted. The central line represents the median of the data and the box edges represent the upper (75th) and lower (25th) percentile. Whiskers denote the highest and lowest values from the data set within the upper and lower limits. Limits are defined as 1.5*50 percentile spread.*

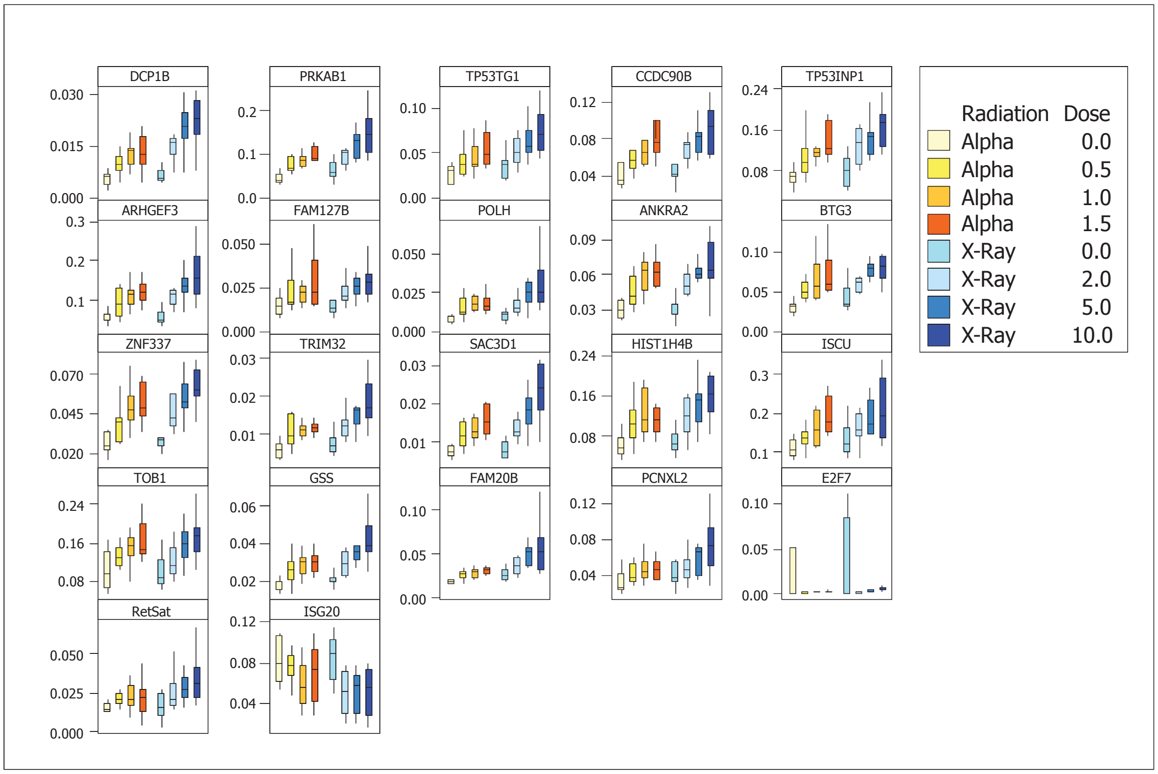


Figure 9: Box-plots of differentially expressed genes assessed using qRT-PCR of cDNA isolated from 12 individuals treated with alpha particle radiation and X-rays. The ***low*** *responding genes that corresponded to the heat-map are plotted. The central line represents the median of the data and the box edges represent the upper (75th) and lower (25th) percentile. Whiskers denote the highest and lowest values from the data set within the upper and lower limits. Limits are defined as 1.5*50 percentile spread.*

4.4 Impact of S & T

4.4.1 Impact and relevance to the identified priority and gap addressed by the project

This community development project is a proof-of-concept initiative and at the early phases of technological development. The initial results seem promising and have potential for operational integration once research towards complete validation of the technology has been undertaken. Specifically, the currently identified gene biomarker panel should be assessed for their ability to produce a robust response in a wider population of individuals. The specificity of the gene panel should be assessed using other radiation types and stressors. Lastly, the dynamic range of sensitivity should be determined, thereby making it practical for use in a large-scale radiological/nuclear event. Once the technology is deemed successful, it will have tremendous impact and relevance to the identified CSSP priority areas. It will support current triage capabilities following a radiological/nuclear (R/N) event or nuclear accidents such as Fukishima. It will help to identify individuals that have been exposed and will provide medical personnel biological dose estimates needed for proper medical intervention. Ultimately, it will enhance our existing bio-nuclear forensics capabilities and emergency preparedness response plans through the detection of those exposed to alpha-particle emitting radioactive material.

4.4.2 Lessons Learned and implementation plan of the Lessons Learned

The majority of the lessons learned from this project have been technical in nature and include:

1) Improvements in methodology for the isolation of white blood cells from healthy individuals.

2) Streamlining methods for RNA extractions and isolation.

3) Troubleshooting in relation to the robotic PCR platform that has been used for high through-put analysis of PCR plates.

4.4.3 New capabilities, partnerships and networks created through the horizontal work of the project

In the process of developing our gene-based biomarker technology, new international partnerships have developed. We are currently collaborating with a group from Columbia University that have resources and expertise in this area of research. Dr Sally Amundson, based in Columbia University (N.Y) holds a patent on the development of a gene bio-chip for detection of individuals exposed to gamma radiation via a finger-prick of blood. Dr David Brenner (Columbia University, NY) who has a world leading radiation facility well-equipped for radiation exposures will provide use of their microbeam facilities. This work has also led to the formation of collaborators at the Ottawa General Hospital (Dr Shawn Malone and Dr Mitchell Sabloff) for the purposes of validating the ex-vivo responses in patients undergoing alpha particle radiation therapy and total body irradiations.

In the process of mining for blood-based biomarkers, a new technological development was conceived. This technological development involved the identification of biomarkers from fingermarks. The conception of this technology has led to the formation of international collaborations with Dr Simona Francese, a fingermark forensics expert. Dr Francese has recently been funded by the Home Office, UK, to validate and integrate the fingermark technology within the forensic enhancement workflow. In terms of operational support, this technology can easily be integrated within the workflow of the RCMP response teams. We have contacted the RCMP CBRNE Response Team and the National Ports Strategy and they have expressed interest in the development of this technology.

5 Transition and Exploitation

5.1.1 Transition to End Users

As the proposed technology is at early stages of development, no considerable effort has been put forth to transition it to the end-users. Our primary goal has been to determine whether the innovation has potential for maturity. Our laboratory has contacted the appropriate end-users (National Biodosimetry Response Team and the RCMP) to determine their needs should the technology be deemed successful. Once the technology has fully matured, knowledge transfer will transpire through meetings, workshops and through participation in live-field exercises held by the National Biodosimetry Response Team and the RCMP Technical and Protective Operational Facility. Proper implementation of this technology will be taught to traditional forensic laboratories and the NBDRP. Lessons learned and knowledge acquired from these training exercises will be shared through seminars, peer-reviewed publications and presentations.

5.1.2 Follow-On R&D Recommended

The initial work has shown some promise, as it has provided a sub-set of dose-responsive genes to radiation exposure which may provide a means for developing strategies for radiation biodosimetry in humans. However, more work needs to be conducted to further the technology. The gene responses require validation for specificity of response, sensitivity and inter-individual variability. Although, initial validation of these responses has commenced, more follow-on research is recommended in order to fully validate the gene markers and compare them with existing technologies such as the gold standard dicentric assay.

5.1.3 Public Information Recommendations

To date, no work has gone towards informing the public of this technology development, as it is still in the early phases of maturity.

6 Conclusion

This study has identified a panel of gene transcripts that are dose-responsive in the range of 0.5 Gy to 1.5 Gy from ex-vivo irradiated blood lymphocytes exposed to alpha-particle radiation. The expression of these transcripts was validated using two technologies and assessed in 12 individuals. The results from this validation allowed for the development of a customized gene array panel which could be used for determining radiation exposure. This gene panel was assessed for its ability to respond in a broader population of cells (i.e WBC's). The panel of genes could easily distinguish un-irradiated and radiated cells with potential for dose estimates (Figure 10). However the panel of genes could not easily distinguish radiation quality, as similar gene responses were obtained for the alpha-particle and X-irradiated samples. No exclusive gene sub-sets between alpha-particle and X-rays were readily expressed with high fold expression levels. However, as the doses were not matched between the two radiation types, it may be plausible to distinguish responses based on intensity/degree of expression. Future work will focus on computational formulations to mine for differences between the expression profiles obtained for the two radiation types. The gene panel will also be further validated for specificity of response in patients undergoing Alpharadin therapy and total body irradiations. Lastly, the sensitivity of the gene panel will be determined for dose and time-points.

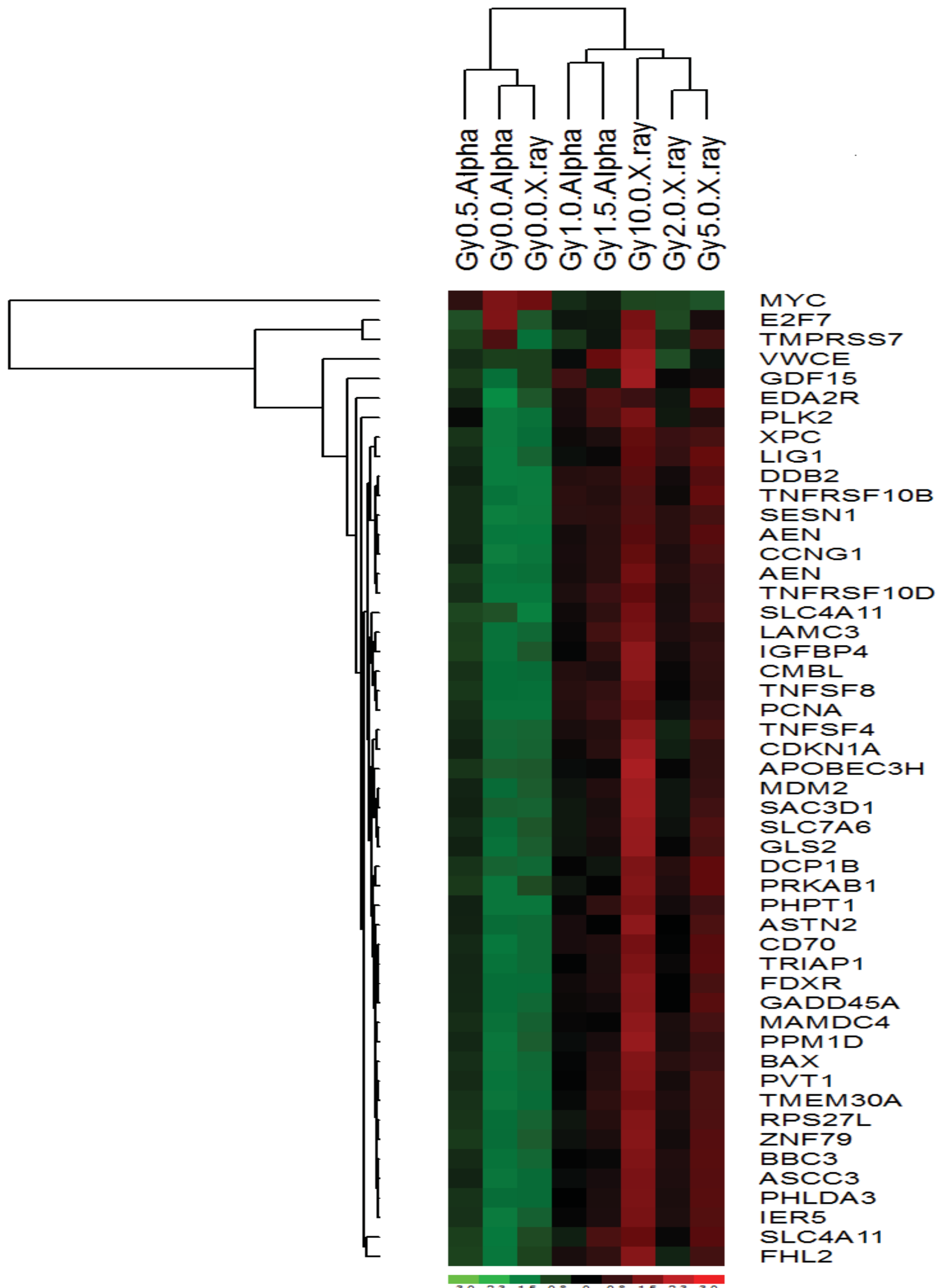


Figure 10: Hierarchical clustering of averaged means of dataset to determine common groupings of samples (distance is measured as 1-correlation and linkage method: average) using dChip (http://www.hsph.harvard.edu/cli/complab/dchip/).

References

Ainsbury, E. A., E. Bakhanova, J. F. Barquinero, M. Brai, V. Chumak, V. Correcher, F. Darroudi, P. Fattibene, G. Gruel, I. Guclu, S. Horn, A. Jaworska, U. Kulka, C. Lindholm, D. Lloyd, A. Longo, M. Marrale, O. Monteiro Gil, U. Oestreicher, J. Pajic, B. Rakic, H. Romm, F. Trompier, I. Veronese, P. Voisin, A. Vral, C. A. Whitehouse, A. Wieser, C. Woda, A. Wojcik and K. Rothkamm (2011). "Review of retrospective dosimetry techniques for external ionising radiation exposures." Radiat Prot Dosimetry 147(4): 573-592.

Anderson RM, Stevens DL, Sumption ND, Townsend KM, Goodhead DT, Hill MA. (2007). Effect of linear energy transfer (LET) on the complexity of α-particle-induced chromosome aberrations in human CD34+ cells. Radiation Research 167(5):541-50.

Andreo, P., M. Evans, J. Hendry, J. Horton, J. Izewska, B. Mijnheer, J. Mills, M. Olivares, P. Ortiz López and W. Parker (2005). "Radiation oncology physics: a handbook for teachers and students." INTERNATIONAL ATOMIC ENERGY AGENCY VIENNA.

Beaton, L., A.; Burn, T., A.; Stocki, T., J.; Chauhan, V.; Wilkins, R..; C. (2011) Development and characterization of an in vitro alpha radiation exposure system. Phys Med Biol., 56, 3645-3658.

Benjamini, Y.; Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society B, 85, 289-30.

Bolstad, B. M. Irizarry, R., A. Astrand, M.;Speed, T., P. (2003) A Comparison of Normalization Methods for High Density Oligonucleotide Array Data Based on Bias and Variance. Bioinformatics. 19, 185-193.

Boyum A. (1968). Separation of leukocytes from blood and bone marrow. Scand. J Clin Lab Invest 21: suppl 97:77.

Chauhan V, Howland M, Chen J, Kutzner B and Wilkins RC. (2011) Differential effects of alpha particle radiation and X-irradiation on genes associated with apoptosis. Radiology Research and Practice.Volume 2011,

Chauhan V, Howland M , Mendenhall A, O'Hara S, ,Stocki TJ, Qutob S, McNamee JP, and Wilkins RC. Effects of Alpha Particle Radiation on Gene Expression in Human Pulmonary Epithelial Cells. International Journal of Hygiene and Environmental Health. 215:522-35.

Chauhan V, Howland M, O'Hara S, Kutzner B, Ferrarotto C, McNamee JP, Bellier PV, Stocki TJ, and Wilkins RC. (2012) Biological Effects of Alpha Particle Radiation in a Human Monocytic Cell-Line. International Journal of Hygiene and Environmental Health. 215(3):339-44.

Chauhan V, Howland M, and Wilkins RC. (2012). A comparative assessment of cytokine expression in human derived cell-lines exposed to alpha particles and X-rays. The Scientific World Journal. Volume 2012, Article ID 609295.

Chauhan V, Howland M, and Wilkins RC. (2012) Effects of α-Particle Radiation on MicroRNA Responses in Human Cell-Lines. Open Biochem J. 6:16-22.

Chauhan V, Howland M. (2012) Genomic Profiling of a Human Leukemic Monocytic Cell-Line (THP-1) Exposed to Alpha Particle Radiation. The Scientific World Journal. (2012), 205038, 2012:8.

Chauhan V, Howland M, Boulay-Greene Hand Wilkins RC. (2012) Transcriptional and Secretomic Profiling of Epidermal Cells Exposed to Alpha Particle Radiation. Open Biochem J. 6:103-115.

Du, P.; Kibbe, W., A.; Lin, S., M.; lumi: A (2008) Bioconductor package for processing Illumina microarray. Bioinformatics, 24, 1547-1548.

Friedrich, T., U. Scholz, T. ElsäSser, M. Durante and M. Scholz (2012). "Systematic analysis of RBE and related quantities using a database of cell survival experiments with ion beam irradiation." Journal of radiation research.23, 123-129.

Franken, N., R. ten Cate, P. Krawczyk, J. Stap, J. Haveman, J. Aten and G. Barendsen (2011). "Comparison of RBE values of high- LET α-particles for the induction of DNA-DSBs, chromosome aberrations and cell reproductive death." Radiation Oncology 6(1): 64.

Goodhead, D. (1994). "Initial events in the cellular effects of ionizing radiations: clustered damage in DNA." International journal of radiation biology 65(1): 7-17.

IPA Calculating and interpreting the p-values for functions, pathways, and lists in Ingenuity Pathways Analysis, (2009) Ingenuity Systems, www.ingenuity.com.

IPA Network Generation Algorithm (2005) Ingenuity Systems, www.ingenuity.com.

Jostes RF.1996. Genetic, cytogenetic, and carcinogenic effects of radon: a review. Mutation Research 340(2-3):125-39.

Kadhim, M., M. Hill and S. Moore (2006). "Genomic instability and the role of radiation quality." Radiation protection dosimetry 122(1-4): 221-227.

Lorimore, S., D. Goodhead and E. Wright (1993). "Inactivation of haemopoietic stem cells by slow α-particles." International journal of radiation biology 63(5): 655-660.

Livak KJ, Schmittgen TD. (200)1. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408.

MacPhail SH, Banath JP, Yu TY, Chu EHM, Lambur H, Olive PL. (2003). Expression of phosphorylated histone H2AX in cultured cell lines following exposure to X-rays. International Journal of Radiation Biology 79 (5):351-358.

National Research Council: Health effects of exposure to radon (BEIR VI). Washington, DC: National Academy Press, 1999.

Nikjoo, H., P. O'Neill, M. Terrissol and D. T. Goodhead (1999). "Quantitative modelling of DNA damage using Monte Carlo track structure method." Radiat Environ Biophys 38(1): 31-38.

Nikjoo, H., P. O'Neill, W. E. Wilson and D. T. Goodhead (2001). "Computational approach for determining the spectrum of DNA damage induced by ionizing radiation." Radiat Res 156(5 Pt 2): 577-583.

Smyth, G., K.; (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Statistical Applications in Genetics and Molecular Biology, 3, Article 3

Smyth, G., K.; (2005) Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions using R and Bioconductor, R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds.), Springer, New York, pages 397-420.

Thomson E, Filiatreault A, Siddiqui Y, Vincent R. (2008). Development and validation of a high-throughput approach for gene expression and toxicity screening. Society of Toxicology of Canada. Montreal, Dec. 1-2, 2008.

Van Tuyle, G., T. Strub, H. O'Brien, C. Mason and S. Gitomer (2003). Reducing RDD concerns related to large radiological source applications. Los Alamos, NM: Los Alamos National Laboratory, LA-UR-03-6664.

Vral, A., M. Fenech and H. Thierens (2011). "The micronucleus assay as a biological dosimeter of in vivo ionising radiation exposure." Mutagenesis 26(1): 11-17.

Annex A Project Team

Position	Name	Title	Phone Number	E-Mail Address
Project Role				
Project Champion	Ms B. Pieterson	Director General, Research and Environmental and Radiation and Health Sciences Directorate (ERHSD), Healthy Environments and Consumer Safety Branch (HECSB), Health Canada (HC)	613-954-3859	Beth.Pieterson@hc-sc.gc.ca
Project Manager/Executor	Dr. V. Chauhan	Research Scientist, CCRPB, ERHSD, HECS, HC	613-941-8516	Vinita.Chauhan@hc-sc.gc.ca
Portfolio Manager	Mr. Ian Summerell	RN Portfolio Manager/CRTI	613-943-2504	Ian.Summerell@drdc-rddc.gc.ca
Project Executor	Mr. M. Howland	Graduate Student, CCRPB, ERHSD, HECS, HC	613-292-1447	Matthew.Howland@hc-sc.gc.ca

Annex B PROJECT PERFORMANCE SUMMARY

PROJECT PERFORMANCE SUMMARY

Technical Performance Summary:

1. Gene biomarkers of radiation exposure have achieved a TRL of 2.5. However further validation of these markers is needed for robustness, sensitivity and inter-individual variability.

Schedule Performance Summary:

Milestone	Project Charter Planned Date	Actual or Forecast	Notes
Project Approval	March 2012	June 2012	Complete
Isolation of peripheral blood mononuclear (PBMN) cells from blood drawn from healthy individuals	May 2012	July 2012	Complete
Exposure of PBMN cells to alpha particles followed by RNA extractions/quantization/ integrity determination	Oct 2012	Nov 2012	Complete
RNA samples out-sourced for microarray analysis (n=5, 4 doses, 1 radiation type)	Jan 2012	Jan 2008	Complete
Statistical analysis /validation of data using alternate technology (qRT-PCR) and pathway analysis.	Feb 2012	Feb 2012	Complete

Cost Performance Summary:

		FY 1	TOTAL
		12/13	
CRTI Funds	CP	88,500.00	88,500
	Spent	86941.41	86941.41
	Difference	1558.59	1558.59
In-Kind	CP	29,205	29,205
	Actual	29,205	29,205
			116,146.41

Overall in-kind contribution was 33%

Annex C Publications, Presentations, Patents

Publications:

Chauhan V, Howland M, Boulay-Greene Hand Wilkins RC. Transcriptional and Secretomic Profiling of Epidermal Cells Exposed to Alpha Particle Radiation. (2012) Open Biochem J. 6:103-115.

Conference Proceedings:

Chauhan V, Howland M, and Wilkins RC. Transcriptional Profiling of Epidermal Cells Exposed to Alpha Particle Radiation for the Development of Biological Assessment Tools. STO-MP-HFM-223, 10:1-12. https://www.cso.nato.int/Pubs/rdp.asp?RDP=STO-MP-HFM-223

List of symbols/abbreviations/acronyms/initialisms

R/N	Radiological/Nuclear
SNM	Special Nuclear Material
PBMN	Peripheral Blood Mononuclear Cells
WBC	White Blood Cells
PCR	Polymerase Chain Reaction
qRT-PCR	Quantitative Real-Time PCR
RDD	Radiological Dispersal Device
LET	Linear Energy Transfer
DNA	Deoxyribo Nucleic Acid
FBS	Fetal Bovine Serum
PBS	Phosphate Buffered Saline
CBC	Complete Blood Count
TBS	Tris Buffered Saline
FSC	Forward Side Scatter
SSC	Side Scatter
IPA	Ingenuity Pathway Analysis
ANOVA	Analysis of Variance
LIMMA	Linear Models for Microarray Data

Glossary

Biodosimetry

Biological dosimetry is a method of measuring the amount of ionizing radiation dose received by an individual using biological materials.

Cytogenetic

The study of the structure of chromosomes.

Functional Genomics

Functional genomics is a field of molecular biology that attempts to make use of the vast wealth of data produced by genomic project

Gamma-H2AX

Marker of DNA damage.

Special Nuclear Material

Defined by Title I of the Atomic Energy Act of 1954 as plutonium, uranium-233, or uranium enriched in the isotopes uranium-233 or uranium-235.

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